Determining the Unknown in Southern Ocean Squid:

Distribution and Diet of *Histeoteuthis eltaninae* and *Martialia hyadesi*

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and to the best of my knowledge, contains no material previously published or written by another person, except where due referencing is made in this thesis.

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ABSTRACT

In the Southern Ocean, cephalopods are infrequently captured by conventional sampling methods, but this is not a reflection of their abundance. This inadequacy has resulted in their being limited knowledge into cephalopods despite their importance as voracious predators and a key prey resource to apex predators. These predators have allowed for a new technique to be developed through using their stomach contents to obtain cephalopods resistant mouthparts (known as beaks). In the Southern Ocean, juvenile elephant seals have the greatest diving and foraging capacity, resulting in the greatest representation of the cephalopod community in their stomach contents. These beaks are morphologically unique to a species, with the dimensions related to the size the whole animal, allowing for species specific knowledge that can be related to size. Diet, potential prey and distribution can be determined through stable isotope analysis.

Stable isotope analysis was conducted on two predominate species found in the stomach contents of juvenile elephant seals at Macquarie Island. *Histioteuthis eltaninae* was the most predominate cephalopod species and is believed to have a life span of approximately one year. Over this year period, $\delta^{13}C$ signatures indicated that there was no active migration occurring, resulting in all life stages inhabiting the same broader region. *H. eltaninae* distribution is relatively confined to waters around Macquarie Island and appears to be restricted by temperature. The $\delta^{15}N$ value indicated a positively linear relationship between trophic level and size of the individual. However, as expected in an annual species, this relationship was effected by season. Despite these relationships, the most predominant prey item appears to correspond with myctophid fish.

Martialia hyadesi was also investigated and appears to have a minimum life span of 16 months indicated by the two concurrent size classes occurring between September and December. The stable isotope analysis demonstrated a broad distribution that involved extensive migration from southern, almost Antarctic waters to waters north of Macquarie Island. Therefore this species must withstand a great diversity of temperatures, particularly when compared to *H. eltaninae*. When considering trophic level and potential prey resources, there appears to be a shift from one tropic level, such as crustaceans and myctophid fish when individuals were smaller, to larger fish and other cephalopods, approximately the next trophic level up in larger individuals. *M. hyadesi* appear to utilize lower trophic levels to a greater extent than *H. eltaninae*, potentially the result of *M. hyadesi* opportunistic nature.

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1. INTRODUCTION

Cephalopods have a significant role in the marine ecosystem. This can be attributed to their biomass, with an estimated standing stock between 193 to 371 million tonnes worldwide (Stowasser *et al.* 2006). This allows them to be a prey source for a diverse array of top predators including marine mammals, seabirds and large pelagic fish. Such a high biomass of cephalopods also has a top-down effect on lower trophic levels. They are able to be voracious, active and opportunistic predators due to their complex sensory structures and powerful muscular arms, which can capture prey items as large as themselves (Jackson 1993; Rodhouse 1989).

In the Southern Ocean, epipelagic fish are largely absent from the food web and have been replaced by the cephalopod community (Rodhouse and White 1995). The absence of epipelagic fish may be a consequences of the physiological constraints that the Southern Ocean imposes on its inhabitants (Rodhouse and White 1995). A cephalopods life history however, has several advantages which may not have constrained this group in the same manner. They typically have a short life cycle, ranging from a few months to two years, with fast growth (Rodhouse and White 1995). They are also semelparous, which allows for a high investment in a relatively large amount of offspring, depleting themselves in the prolonged reproductive process and eventually dying of starvation (Rodhouse and White 1995). Due to their short life cycle, the planktonic to nektonic phase (if present) are relatively brief, allowing them to stay in the productive regions before being transported by the Antarctic Circumpolar Current (Rodhouse and White 1995).

Despite cephalopods being a key group in the Southern Ocean food web, there is limited fundamental knowledge on these species (Cherel *et al.* 2004). There are several reasons for this, predominantly the difficulty of obtaining whole specimens by conventional sampling methods such as nets, due to their strong swimming capability (Nemoto *et al.* 1985). Furthermore, sampling can typically only occur during the ice-free summer periods (Cherel *et al* 2004; Cherel and Hobson 2005; Groger *et al.* 2000). While there is difficulty in obtaining specimens, this is not a true reflection of their relative abundance, biomass and ecological importance in the Antarctic (Clarke 1996).

Determining the diet of cephalopods can also be difficult and is not confined to the Southern Ocean. Cephalopods macerate their prey beyond the point of being identifiable due to their narrow oesophagus (Rodhouse 1989; Ruiz-Cooley *et al.* 2006). Therefore hard remains are required to identify their prey. However, cephalopods often reject these hard structures due to this narrow oesophagus (Rodhouse 1989; Ruiz-Cooley *et al.* 2006). This is particularly the case for otoliths of large fish, with squid using their beaks to take bites out of these larger prey items (Dickson *et al.* 2004). The fish heads are often discarded, causing the otoliths not to be present in the stomach contents (Rodhouse *et al.* 1992b). Stomach contents can also be influenced by secondary digestion, where the prey items in the stomach of the consumed are incorporated into the stomach contents the predator (Dickson *et al.* 2004).

Consequently, alternative methods for sampling Southern Ocean cephalopods are needed, such as utilizing the stomach contents of predators. Cephalopods have resistant, indigestible mandible (known as beaks) which accumulate in the stomachs of their predators. While the analysis of stomach contents can yield information into the predator and ecosystem interactions, it can also provide considerable information into the cephalopod species themselves. Predators are able to catch a greater diversity of squid species and size ranges than conventional sampling methods. Such investigations can be taken further with stable isotope analysis, which allows for insight into their trophic position, possible prey resources and general distribution.

Cephalopod beaks are morphologically unique to a species, and their dimensions can be used to estimate the size of individual animals (Lu and Ickeringill 2002). The lower rostral length is commonly used to determine the size of the individual, either in mantle length or mass (Groger *et al.* 2000). The relationships between the size of the individual and lower rostral length do not differ significantly between the sexes or as an individual matures (Bolstad 2006). When there are several individuals present in a predators stomach contents, the biomass of the cephalopod community consumed can be yielded (Groger *et al.* 2000; Lu and Ickeringill 2002).

While beaks can determine the length of an individual, there is considerable debate over the estimation of age from size in cephalopods. Age is required to determine life histories such as growth and longevity. Originally, squid growth was estimated through length-frequency data

using the von Bertalanffy growth curve developed to describe fish growth (Dawe and Beck 1997). However, statolith aging techniques demonstrated a brevity cephalopod life-span, ranging from a few months to two years, with fast consistent growth occurring during this time (Jackson *et al.* 1997; Oosthuizen 2004).

Estimating age from length is also confounded by a cephalopods growth which is strongly influenced by several biotic and abiotic factors, predominately water temperature and food availability (Oosthuizen 2004). With a short life span, these environmental influences cause different growth rates between generations. This plasticity in growth allows cephalopods to take advantage of patchy resources, resulting in a boom of growth when conditions are favourable. However, these same factors cause a cephalopod to be unable to store reserves which are needed to survive infertile periods (Jackson *et al.* 2005). These unfavourable conditions result in a crash of the cephalopod population (Jackson *et al.* 2005). The combination of a short life span, extended spawning season and their rapid growth rates which are strongly influenced by environmental parameters result in a complex population structure that is relatively unpredictable (Jackson *et al.* 2005). The consequence is large variations in growth for cephalopods, both on a large temporal and spatial scales, but also in a given population (Oosthuizen 2004). A prolonged spawning period is characteristic in several cephalopod populations. The environmental conditions differ for the individuals born at the start of the season to those that hatch at end of the spawning period. Therefore, the growth rates of a given cohort will differ. This makes the extrapolation of age from size biased and therefore inappropriate.

Stable isotope analysis is commonly used to investigate the nitrogen ($^{15}N/^{14}N$, measured as $\delta^{15}N$) and carbon ($^{13}C/^{12}C$, $\delta^{13}C$) ratios in animal tissue. These two isotopes indicate trophic level and broad distribution respectively, and combined, can indicate potential prey resources. Isotopes can be measured due to inorganic substrates (such as carbon and nitrogen) being incorporated into phytoplankton and particulate organic matter (POM) by photosynthesis processes (Hobson and Cherel 2006; Porras-Peters *et al.* 2008). The isotope values, predominately carbon, vary spatially, due to primary producers differing regionally as the result of environmental factors and growth rates (Porras-Peters *et al.* 2008). The plankton food base at higher latitudes are more enriched with $\delta^{13}C$ when compared to equatorial communities (Cherel and Hobson 2007). In the southern hemisphere, the geographic $\delta^{13}C$ gradient is well

defined with high $\delta^{13}C$ values in the cold southern Antarctic waters and decreasing $\delta^{13}C$ the closer the specimen is distributed to the tropics and can therefore be used to investigate the foraging regions of marine predators (Cherel and Hobson 2007).However, it has been suggested that this relationship is not linearly related to latitude (Cherel and Hobson 2007). It has been proposed that there is little deviation in the plankton food base, indicated by the carbon signature, in a given water mass and instead, there is abrupt changes at ocean fronts, resulting in a stepwise relationship (Cherel and Hobson 2007). This indicates that there is a relatively consistent carbon isotope value that is found across the Antarctic Zone, but an abrupt increase in ^{13}C across the Polar and Subantarctic Front as well as the Subtrophic Front (Cherel and Hobson 2007). With these conflicting observations, the interpretation of the carbon stable isotope composition should be regarded on a large geographical scale of water masses and fronts (10s to 100s of km in latitude) (Cherel and Hobson 2007).

In regards to nitrogen, there are changes that occur as the isotope moves through the trophic levels of a food web. These changes are relatively consistent and an isotopes behaviour through an ecosystem can be predicted (Hobson and Cherel 2006). $\delta^{15}N$ levels demonstrate an average stepwise enrichment between 2.5-3.4 ‰ with each of the changes in trophic level (Cherel and Hobson 2005; Stowasser *et al.* 2006). The differences in the $\delta^{15}N$ levels between individuals allow for the assessment of a species' position in a food web.

Stable isotope analysis on squid beaks can be a useful technique for investigating the feeding ecology and distribution of cephalopods (Ruiz-Cooley *et al.* 2006). Being metabolically inert, beaks can reflect an individual's diet throughout their life history (Ruiz-Cooley *et al.* 2006). Cephalopods beaks grow through the accretion of new molecules (predominately proteins and chitin) from the rostral tip and build on the recently formed wings and lateral walls (Cherel and Hobson 2005). Consequently, the cephalopod beaks retain molecules from their early development (the rostral tip) to the time of their death (the lateral wall and wings).

Stable isotope analysis needs to be conducted on lipid free material and a cephalopod beak contains approximately 90% protein and approximately 10% chitin (Hobson and Cherel 2006). However, it is this chitin material that causes a depletion in the $\delta^{15}N$ isotope value when compared to lipid-free muscle tissue as well as their prey material (Cherel *et al.* 2009; Xavier *et al.* 2007). However, this can be accounted for through the correction of the nitrogen isotope

value by approximately 3 ‰ if comparison between these materials is required (Cherel *et al.* 2009). The biochemical composition of the wing can vary with coloration also. Clear or undarkened young wings have a higher proportion of chitinous material when compared to darkened, older wings (Cherel *et al* 2009). With chitin being impoverish to the ^{15}N isotope, this results in a lower $\delta^{15}N$ value and higher C/N rations than what it should be (Cherel *et al.* 2009). Consequently, clear wings, which are predominately small young individuals, cannot be compared to older, darkened wings without confounding the $\delta^{15}N$ signature and therefore the interpretation of trophic position.

However, there are limitations to stable isotope analysis in that they offer less detailed information on dietary composition than traditional method such as stomach contents and scat analysis. The carbon and nitrogen ratios, however, can be extrapolated to determine potential food resources, but need confirmation through traditional techniques (Porras-Peters *et al.* 2008). The use of stable isotope analysis does overcome the biases that can occur when traditional methods are used alone. Stomach contents only give an indication of prey resources over a short time period. The advantage of stable isotope analysis is that it can provide a record of trophic level and potential prey items throughout the life time of the individual (Hückstädt *et al.* 2007). This is of particular importance when whole specimens are difficult to capture and therefore stomach contents do not give an adequate depiction of the prey consumed by a population.

With conventional sampling methods obtaining inadequate sample sizes of cephalopods, resulting in a lack of fundamental knowledge on these populations, an important supplementary method will be through the stomach contents of their predators. Southern elephant seals offer a particularly rich source of information. Elephant seals dive to more 1,900 m, with an average diving depth between 300 to 600 m (van den Hoff 2004). These seals are also able to travel large distance when foraging, which are largely dominated by squid (Field *et al.* 2007).

This current study utilized the stomach contents of southern elephant seals from Macquarie Island used by Field *et al.* (2007). A diversity of squid beaks dominated the seals stomach contents, allowing for an assortment of squid species to be investigated. *Histioteuthis eltaninae* was the most numerous prey item for this population of seals and are an important

prey resource to numerous apex predators in the Southern Ocean (Field *et al.* 2007). Despite their importance, there is limited knowledge into this species beyond their predation by apex predators. *Martialia hyadesi* is also a key prey item for numerous higher order predators throughout Antarctic waters, including the elephant seals at Macquarie Island (Field *et al.* 2007). This species has the potential for commercial harvest with exploratory fishing operations already occurring in South Georgia (Kock 2000; 2001). However, the knowledge obtained from such explorations is largely limited to stomach content analysis (Rodhouse *et al.* 1996; Rodhouse *et al.* 1992b). Currently there is no information on the south Pacific Ocean populations of this species. With increased interested into a fisheries for *M. hyadesi*, it is important to determine life history knowledge into the species itself, as well as their interactions with the surrounding ecosystem as required by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (Kock 2000; Rodhouse and White 1995). The aim of this study is to investigate the trophic position, prey and broad distribution of *H, eltaninae* and *M. hyadesi* for the Macquarie Island population through the stable isotope analysis technique.

2. MATERIALS AND METHODS

2.1 Stomach contents

Between November 1997 and December 2000, 468 southern elephant seals (*Mirounga leonine*) stomachs were flushed with sea water, as outlined by Field *et al.* (2007), when returning to Macquarie Island (158° 57'E, 54° 30'S). The flushing procedure was repeated three times on an individual in order to remove the majority of the stomach contents. The regurgitation was filtered through a 1 mm sieve and once filtered, the stomach contents were placed into a storage jar filled with 70% ethanol until the stomach contents were sorted and the prey items identified.

When the stomach contents were sorted, the squid mouth parts (both the upper and lower sections) were separated from the other contents. The lower squid beaks were identify to the lowest taxa possible with the aid of Clarke (1986) and reference specimens (from Malcolm

Clarke held at the Australian Antarctic Division). The lower rostral lengths were measured to ± 0.01 mm.

From these stomach contents there were 9,232 individual squid beaks from 31 identified species (and 145 individuals that could not be identified). The beaks were stored in jars based on the stomach contents that they had been collected from and filled with 70% ethanol.

2.2 Cohort analysis

For the predominate species in the stomach contents, histograms of each month were used to examine two factors. Firstly, to determine if the squid the seals had been predating on represented a normal or skewed size distribution during the sampling period for a particular species. Secondly, to examine how the size classes (or size cohorts) of these sampled beaks progressed through time. For squid species that have a life span of one year, there are no overlapping cohorts, resulting in just a single peak for every month being observed during the 12 month period. However, when a species has a life span of more than one year, then the histogram will display two peaks for consecutive month, when a species has a life span of one and two years, three peaks for a species that has a life span between two and three years and so on.

2.3 Stable isotope analysis

Stable isotope analysis were conducted on a sub sample of two predominate species found in the stomach contents, *Histioteuthis eltaninae* and *Martialia hyadesi*. *H. eltaninae* was the most common species with 3,568 beaks in total (38.6% of all squid beaks sampled). *M. hyadesi* was the fifth abundant species with 559 beaks represented (6% of the squid beaks). The size class cohort analysis for *M. hyadesi* demonstrated two overlapping size classes between September and December.

For these two species, six lower beaks were randomly selected from each months (with the months pooled over the three years). The months that did not contain six beaks but still had two beaks that could be sampled, all the beaks in that month were selected. For *M. hyadesi*,

two separate size class cohorts occurred between September and December. The beaks that had a lower rostral length larger than 7.0 mm where classified as cohort one, where those smaller than 7.0 mm were classed as cohort two. In order to represent both of these size classes, six beaks were randomly selected for each of these groups over this four month period, resulting in 12 beaks being represented, when they were available.

However, undarkened or clear wings have a greater chitin composition when compared to darkened wings (figure 1) (Cherel *et al.* 2009). Since chitin is impoverish to $\delta^{15}N$ when compared to diet, this results in a higher C/N ratio than what there should be. Due to the higher chitin content of undarkened wings, the $\delta^{15}N$ isotope signature has limitations in their isotope signature and therefore does not indicate the diet of that individual accurately. Therefore, all beaks used for stable isotope analysis required the wing region to have the same colouration, and darkened beaks were chosen as they represented a larger proportion of the population.

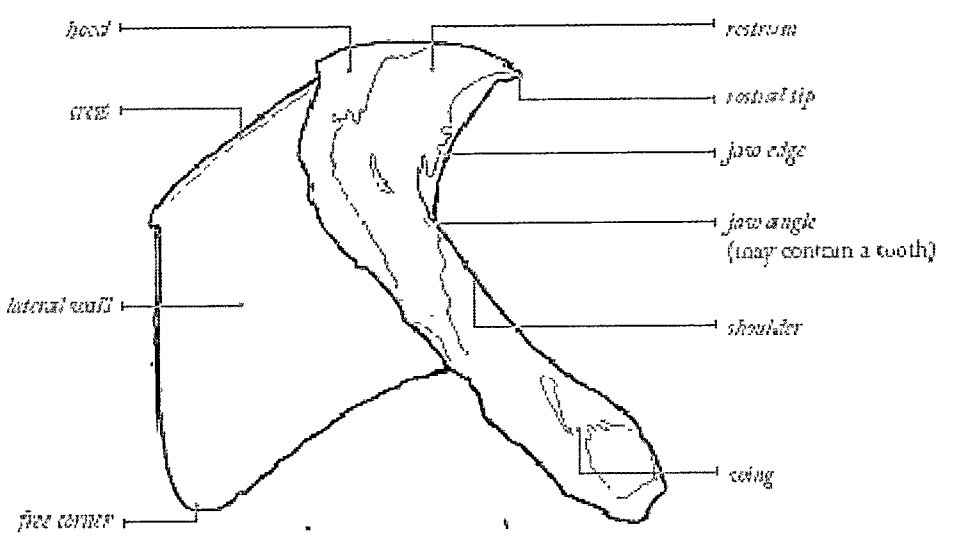

Figure 1 Key features and the principal terms used to characterize decapod beaks (Xavier and Cherel 2009).

The beaks that had been randomly selected where cleaned thoroughly using tweezers and distilled water in order to prevent contamination from the stomach contents. The wings were then cut off using clean scissors and the wings placed into an unused test tube. All equipment

used was cleaned using distilled water, acetone and kimwips in order to prevent cross contamination. The beak wings were then dried in an oven at 60°C for a minimum period of 16 hours.

Once dried, the wings from one individual were ground into a homogenous powder with a maximum particle size of 0.1 mm by using a glass mortar and pestle. This powder was then placed into 4 x 3.2 mm foil capsules which had previously been weighed, and the sample and capsule were weighed again. The weight of the beak sample ranged from 0.2 mg to 2.9 mg. The capsules where then folded and rounded. These enclosed foil samples were then placed in individual containers that were labelled with a unique code. Measures were taken to prevent cross contamination as outlined previously.

When all samples from one species had been processed, they were then sent to the Environmental Biology Group, Stable Isotope Facility at the Australian National University in Canberra, where they determined the $\delta^{15}N$ and $\delta^{13}C$ ratio in the same beak. The beak samples were combusted in a strongly oxidized helium column at1050°C, producing gases. The gases produced were then transported to a reducing furnace tube, kept at 660°C, in order to cool the sample. Magnesium perchlorate absorbed the water, resulting in a dried gas that could then be passed through a gas chromatograph in order to separate the components of interest (nitrogen and carbon). These gases were then transported at known pulses into a Fisons Isochrom Continuous-flow Isotope Ratio Mass Spectrometer where the isotopic composition was determined.

2.4 Analysis

The mantle length (ML) and mass (Wt) was calculated from the lower rostral length (LRL), using the following empirically derived relationships:

Histioteuthis eltaninae (Lu and Ickeringill 2002): $ML = -3.65 + 24.48\ LRL$ ($r^2 = 0.99$, $n = 6$)

$$WtP = 0.33 + 3.11\ InLRL \quad (r^2 = 0.79, n = 5)$$

Martialia hyadesi (Xavier and Cherel 2009): $ML = 102.0 + 29.47\ LRL$ ($r^2 = 0.79$, $n = 67$)

$$InWt = 2.405 + 2.012\ InLRL \quad (r^2 = 0.79, n = 67)$$

A linear regression was conducted on the $\delta^{15}N$ and $\delta^{13}C$ signatures against the size of the squid (lower rostral length, mantle length and mass) in order to determine if a relationship was occurring between the size of the individuals in a given population and the isotope value. For *M. hyadesi*, between September and December, t-tests were conducted to compare the two different concurrent size classes in regards to their $\delta^{15}N$ and $\delta^{13}C$ signatures that were represented over the four month period. A t-test was used to compare the isotope signatures of these two species. A general linear model was also used to compare these two species in regards to their $\delta^{15}N$ and $\delta^{13}C$ ratios as well as the effect of month.

The potential prey of *M. hyadesi* and *H. eltaninae* was also examined. The stable isotope compositions of potential prey resources for these two species were obtained from the literature (Cherel 2008; Cherel *et al.* 2004; Cherel *et al.* 2000; Cherel *et al.* 2002; Cherel and Weimerskirch 1999; Schmidt *et al.* 2004) (table 1). Both species were broken into four equal sample size classes in order to compare the $\delta^{15}N$ and $\delta^{13}C$ ratios with potential prey items. This allowed for the estimation of potential prey of the squid population. However, the high chitin content in the beak results in the nitrogen isotope signature to be dampened when compared to tissue that does not contain this element, such as their potential prey (Cherel *et al.* 2009). In order to compare these two different materials, a correction factor of 3 ‰ is required for the nitrogen values obtained from the beaks (Cherel *et al.* 2009; Cherel and Hobson 2005).

Table 1 Description of the potential prey resources for both *Histioteuthis eltaninae* and *Martialia hyadesi* that have been obtained from the literature.

Species	Label in graphs	Group	Type	Mean $\delta^{13}C$	$\delta^{13}C$ SD	Mean $\delta^{15}N$	$\delta^{15}N$ SD	N	Location	Max. Size
Themisto gaudichaudii	Them.	crustacean	amphipod	-22.8	0.7	5	1.2	10	Kerguelen	
Euphausia superba	E.sup.	crustacean	euphausiid	-25.8	0.4	5.5	0.4	12	Adélie Land	
Euphausia vallentini	E.val.	crustacean	euphausiid	-20	1.2	5.4	0.8	10	Kerguelen	
***Thysanoessa* spp. Juvenile**	Thys.	crustacean	euphausiid	-22.2	0.3	5.3	0.9	3	PF (~49S, 20E)	
***Euphausia frigida* juvenile**	E.frig.	crustacean	euphausiid	-24.3	0.9	4.9	0.3	4	PF (~49S, 20E)	
Krefftichthys anderssoni	Kreff.	fish	myctophid	-22.3	0.2	7.6	0.2	12	Kerguelen	69 (mm)
Electrona antarctica	E.ant.	fish	myctophid	-21.4	0.5	8.9	0.3	12	Kerguelen	85 (mm)
Electrona carlsbergi	E.carl.	fish	myctophid	-21.6	0.4	9.5	0.2	12	Kerguelen	97 (mm)
Electrona subaspera	E.sub.	fish	myctophid	-20.2	0.4	7.3	0.3	14	Kerguelen	117 (mm)
Gymnoscopelus nicholsi	G.nic.	fish	myctophid	-21.1	0.3	10.2	0.5	12	Kerguelen	139 (mm)
Gymnoscopelus fraseri	G.fra.	fish	myctophid	-21.1	0.4	9	0.4	12	Kerguelen	78 (mm)
Protomyctophum tenisoni	P.ten.	fish	myctophid	-22.1	0.3	8.1	0.3	11	Kerguelen	51 (mm)
Dissostichus eleginoides	Disso.	fish	Patagonian toothfish	-19	0.7	12.9	0.6	15	Kerguelen	215 (cm)
Pleuragramma antarcticum	Pleura.	fish	Antarctic silver fish	-24.7	0.4	10.6	0.3	5	Adélie Land	53 (mm) SL
Martialia hyadesi	Mart.	squid	squid	-20.9	0.5	7.7	0.6	10	Kerguelen	
Kondakovia longimana	Kond.	squid	squid	-21.1	0.5	9.2	1.1	3	Kerguelen	757.4 (mm) ML
Histioteuthis eltaninae	Hist.	squid	squid	-20.7	0.4	10.4	0.7	10	Kerguelen	
Psychroteuthis glacialis	Psych.	squid	squid	-25	0.3	10	0.7	10	Adélie Land	380 (mm) ML
Channichthys rhinoceratus	Channich.	fish	ice fish	-19.6	1.5	11.9	1.4	4	Kerguelen	520 (mm)

3. RESULTS

3.1 *Histioteuthis eltaninae*

3.1.1 Size distribution of *Histioteuthis eltaninae*

The most predominate prey species found in the stomach contents of the southern elephant seals (*Mirounga leonine*) at Macquarie Island was *Histioteuthis eltaninae*, totalling 3,568 identifiable beaks. These beaks ranged in size from a lower rostral length of 0.94 mm to 3.94 mm, which corresponds to a calculated mantle lengths of 19.36 mm and mantle length of 92.80 mm respectively. The mean lower rostral length for all of the *H. eltaninae* beaks was 2.574 mm (calculated mantle length of 59.36 mm).

When investigating all of the *H. eltaninae* beaks through time, the smallest mean sized month occurred in July, with a mean mantle length of 45.26 mm (figure 2). January demonstrated the largest mean mantle length of 64.82 mm, which is a three times larger than the smallest mean size month of July. When observing the months consecutively, the greatest increase in the size is between August and September, which demonstrated an increase of almost 10 mm in mantle length. Generally, though, the increase in mean mantle length between months was smaller, ranging from 0.46 mm between September and October and 3.35 mm between October and November.

For the subset *H. eltaninae* beaks that were used in the stable isotope analysis, there was a lower rostral length range from 1.9 mm (mantle length of 42.86) to 3.3 mm (mantle length of 77.13 mm). 71 individuals where used in the stable isotope analysis and represented 2 % of the total amount of *H. eltaninae* beaks found in the stomach contents of the elephant seals. The mean lower rostral length of the beaks used in the stable isotope analysis was 2.6 mm, with an estimated mantle length of 58.73 mm.

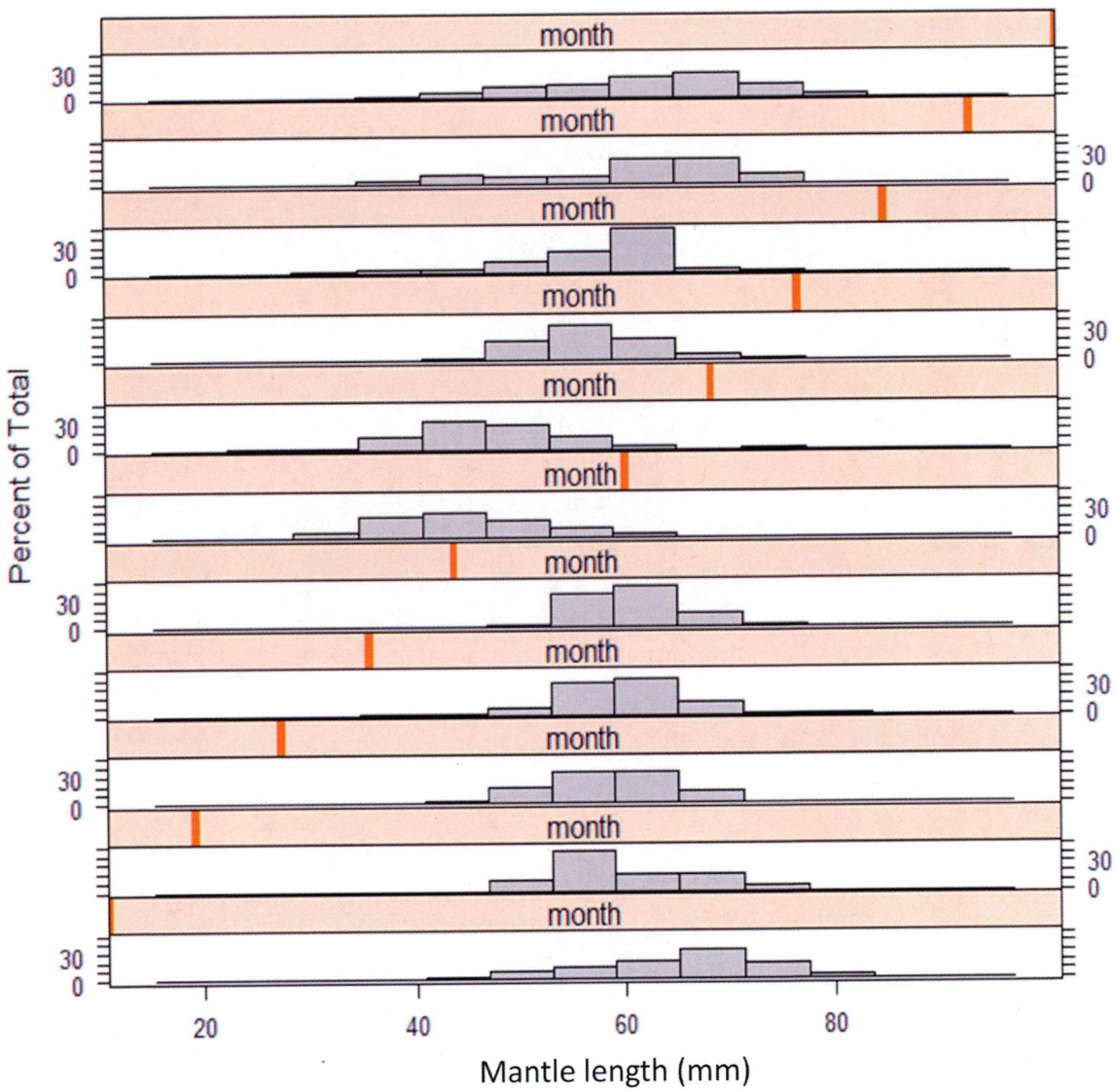

Figure 2 Size distribution (mantle length (mm)) of all *Histioteuthis eltaninae* beaks found the stomach contents of southern elephant seals (n = 3,568) over 11 consecutive months (with no samples to represent June).

3.1.2 Trophic position of *Histioteuthis eltaninae*

The nitrogen isotope signature for *Histioteuthis eltaninae* beaks ranged from 0.22 ‰, which had a lower rostral length of 2.4 mm to 7.72 ‰ (two and a half trophic levels), with a lower rostral length of 3.2 mm. The smallest beak according to lower rostral length (1.9 mm) had a $\delta^{15}N$ signature of 3.1 ‰ (one trophic level). The largest beak (lower rostral length of 3.3 mm) had a nitrogen signature of 6.93 ‰ (two trophic levels). There was a significant relationship between $\delta^{15}N$ and the mantle length of *H. eltaninae* ($r = 0.3212$, $r^2 = 0.283$, $F = 28.63$, $p = < 0.05$) (figure 3).

The results from the stable isotope analysis also had five low values that were less than 3 ‰, which is a single trophic level. These five values, ranging from 0.22 ‰ to 2.05 ‰, all occurred in July and August, with the lower rostral length ranging from 2.2 and 2.5 mm. The beak that had a $\delta^{15}N$ signature of 0.22 ‰ and 0.41 ‰ both had a small clear region on the wing. However, these clear regions were not included in the analysed sample. The remaining beaks had all darkened wings and yet still had a nitrogen signature ranging from 1.51 ‰ to 2.05 ‰. All of these five samples had adequate mass for the stable isotope to be conducted upon them. Therefore there was no reason to exclude these values from the data analysis.

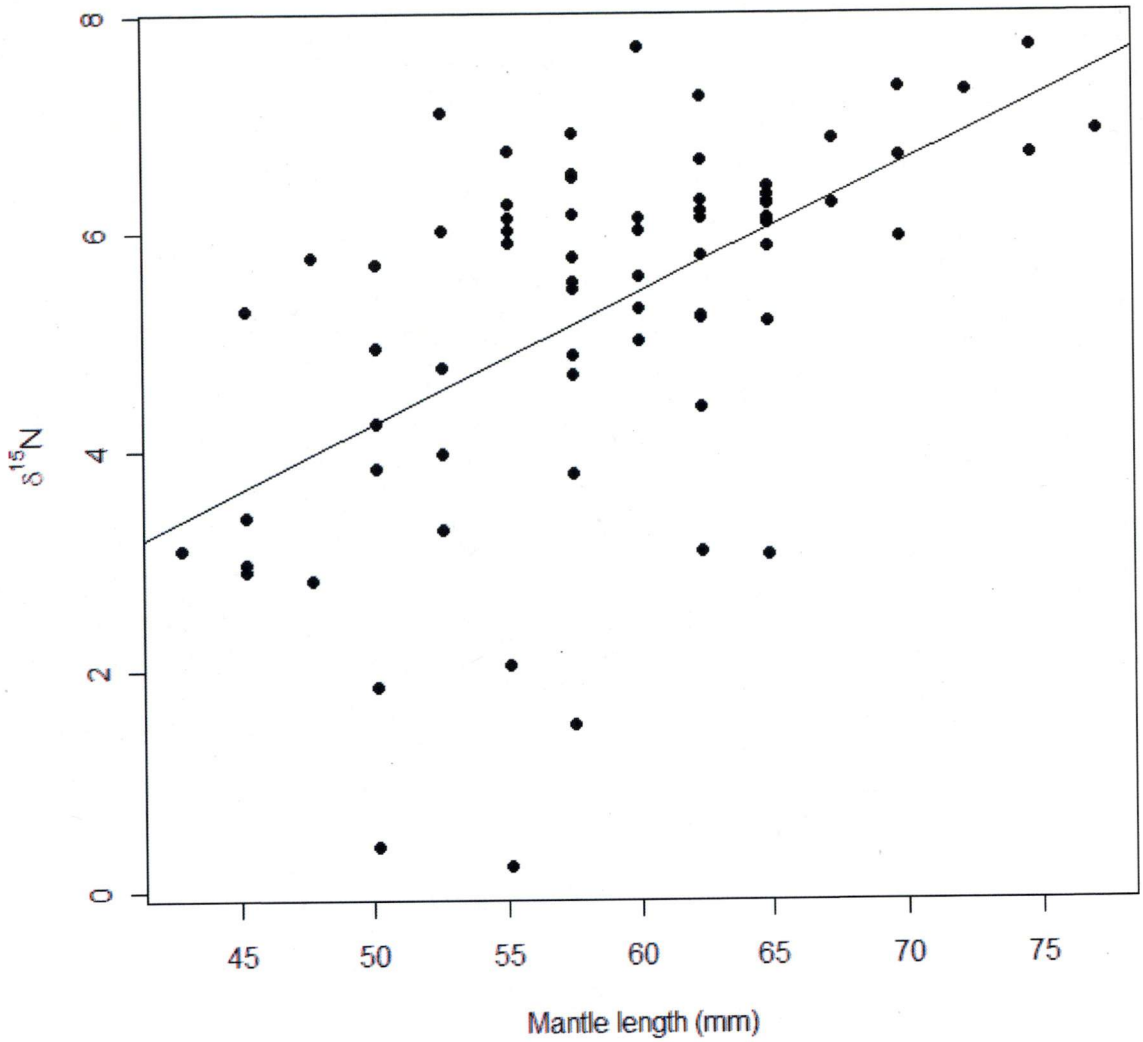

Figure 3 Relationship between $\delta^{15}N$ and calculated mantle length (mm) of *Histioteuthis eltaninae* demonstrated by a scatter plot with a regression line: $y = 0.1208x - 1.8072$ ($r = 0.3212$, $r^2 = 0.283$, $F = 28.63$, $p = < 0.05$; $n = 71$).

While the $\delta^{15}N$ value increased with size of the *H. eltaninae* sampled, this relationship is confounded by the time of year (figure 4). Generally, the smallest individuals occurred between July and September in the randomly selected beaks, with a ranging lower rostral length between 2.0 and 3.2 mm. Smaller sized beaks during this three month period were also reflected in the total amount of *H. eltaninae* collected from the stomach contents (figure 2). The $\delta^{15}N$ values range from 0.41 ‰ to 7.72 ‰ over this three month period. For the remainder of the year, the lower rostral length ranged from 1.9 mm to 3.3 mm.

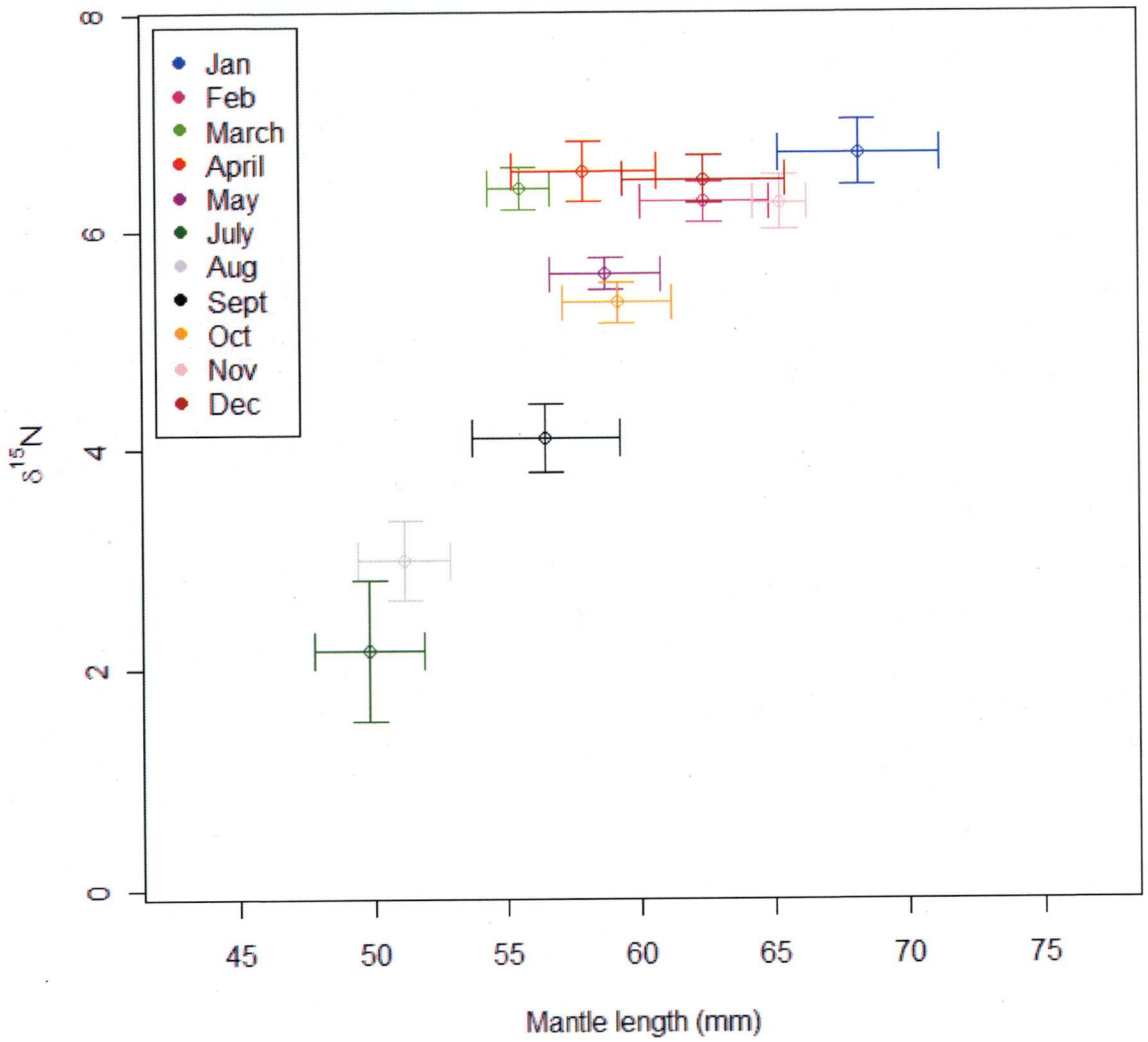

Figure 4 $\delta^{15}N$ and calculated mantle length of *Histioteuthis eltaninae* grouped by month, demonstrating the mean and standard error (n = 71).

3.1.3 Potential prey of *Histioteuthis eltaninae*

The squid *Histioteuthis eltaninae* where grouped into four equal size classes according to the size of the beak (lower rostral length (1.9-2.3 mm, 2.3-2.5 mm, 2.5-2.7 mm, 2.7-3.3 mm)) in order to compare against the stable isotope ratios of their potential prey resources. With a significant relationship occurring between the size of the individual and trophic level, the grouping of size classes allowed for the examination of potential prey shifts with size. The mean $\delta^{15}N$ and $\delta^{13}C$ where determined for these four size classes (table 2). The $\delta^{13}C$ value varied little and ranged from -21.01 ‰ (largest size class) to -21.38 ‰ (the second smallest size class). The mean $\delta^{15}N$ values for these four size class

groups' increases with the size of the grouped individuals, with a range greater than 2 ‰ between the smallest size class to the largest individuals.

Table 2 The mean stable isotope value for each of the size classes of *Histioteuthis eltaninae* used to compare with potential prey. Due to the depletion of $\delta^{15}N$ in cephalopod beaks, the correct nitrogen value (addition of 3 ‰) was used to compare with the potential prey resources.

Size classes (lower rostral length)	Mean of $\delta^{15}N$	Corrected mean of $\delta^{15}N$	Mean of $\delta^{13}C$
1.9-2.3 mm	4.02 ‰	7.02 ‰	-21.29 ‰
2.3-2.5 mm	5.15 ‰	8.15 ‰	-21.38 ‰
2.5-2.7 mm	5.75 ‰	8.75 ‰	-21.28 ‰
2.7-3.3 mm	6.3 ‰	9.3 ‰	-21.01 ‰

When determining potential prey from the $\delta^{15}N$ and $\delta^{13}C$ value of the predator, there are several possible scenarios. The first possibility is that the stable isotope value of the predator could correspond directly to the possible prey item. It is also possible however, that the isotope signature is a mixture of higher and lower $\delta^{15}N$ values than the predator. The likelihood of these various scenarios can be determined through the knowledge that squid are only able to predate on individuals that are as large as themselves.

When comparing the four size classes of *H. eltaninae* to potential prey items that have been found in the literature (table 1), there appears to be a correspondence with myctophid fish and mid-trophic level squid (figure 5). Despite the positive linear relationship that occurs between nitrogen and size of individuals in this population, the four size classes are clumped relatively tightly by both $\delta^{15}N$ and $\delta^{13}C$ signatures.

More specifically, the smallest size class with a lower rostral length between 1.9 to 2.3 mm (corrected $\delta^{15}N$ of 7.02 ‰ and $\delta^{13}C$ -21.29 ‰) aligns with two myctophid species. Firstly, *Krefftichthys anderssoni*, which has a $\delta^{15}N$ value of 7.6 ‰ (approximately 0.6 ‰ more than *H. eltaninae*) and a $\delta^{13}C$ value of -22.3 ‰ (almost 1 ‰ less than the smallest size class of *H. eltaninae*). The other myctophid is *Electrona subaspera* which had a $\delta^{15}N$ signature of 7.4 ‰ (approximately 0.3 ‰ more than *H. eltaninae*) and $\delta^{13}C$ -20.2 ‰

(almost 0.9 ‰ more than the carbon mean signature of the smallest size class of *H. eltaninae*).

The size class with a lower rostral length between 2.3 and 2.5 mm (with a corrected mean $\delta^{15}N$ of 8.15 ‰ and $\delta^{13}C$ of -21.38 ‰) corresponds with *Krefftichthys anderssoni* also, which differs in $\delta^{15}N$ by approximately 0.6 ‰ and $\delta^{13}C$ 1 ‰. Another corresponding prey species is *Protomyctophum tenisoni* which has a $\delta^{13}C$ of -22.1 ‰ (difference of 0.7 ‰ with *H. eltaninae*) and $\delta^{15}N$ of 8.1 ‰ (difference of 0.05 ‰).

The 2.5 to 2.7 mm size class has a mean corrected nitrogen stable isotope value of 8.75 ‰, and carbon value of -21.28 ‰. This size class corresponded with the myctophid *Electrona antarctica* which has a $\delta^{13}C$ of -21.4 ‰ (0.12 ‰ difference with *H. eltaninae*) and $\delta^{15}N$ value of 8.9 ‰ (difference of 0.15 ‰). The myctophid *Gymnoscopelus fraseri* has a $\delta^{13}C$ of -21.1 ‰ (*H. eltaninae* is 0.18 ‰ less than this myctophid) and $\delta^{15}N$ of 9 ‰ (difference of 0.25 ‰).

The largest size class of *H. eltaninae* has a lower rostral length between 2.7 and 3.3 mm, which has a corrected nitrogen stable isotope value of 9.3 ‰, and carbon value of -21.01 ‰. This correlates with the myctophid *Electrona carlsbergi* which has a $\delta^{13}C$ of -21.6 ‰ (approximately 0.6 ‰ difference with *H. eltaninae*) and $\delta^{15}N$ of 9.5 ‰ (difference of 0.2 ‰).

It appears that myctophid fish are the predominate prey resource that *H. eltaninae* feed on. While it is possible that *H. eltaninae* obtain their nitrogen and carbon ratios by feeding on a mixture of higher and lower trophic levels than the corresponding myctophid fish, this appears to be unlikely. Squid are only able to feed on individuals that are as large as themselves. The largest *H. eltaninae* used in the stable isotope analysis had a mantle length of 58.73 mm. Therefore it is unlikely that this population is feeding on the ice fish, *Channichthys rhinnoceratus*, a species that can reach a maximum size more than 5.5 times larger than the largest specimen of this squid. The same principle applies to the squid species *Kondakovia longimana*, which can reach sizes over 750 mm in mantle length and the Patagonian toothfish which can reach sizes over two meters. Therefore, the most likely scenario in regards to prey items of *H. eltaninae* is that they feed predominately on myctophid fish that inhabit the same approximate water mass as indicated by the carbon isotope value.

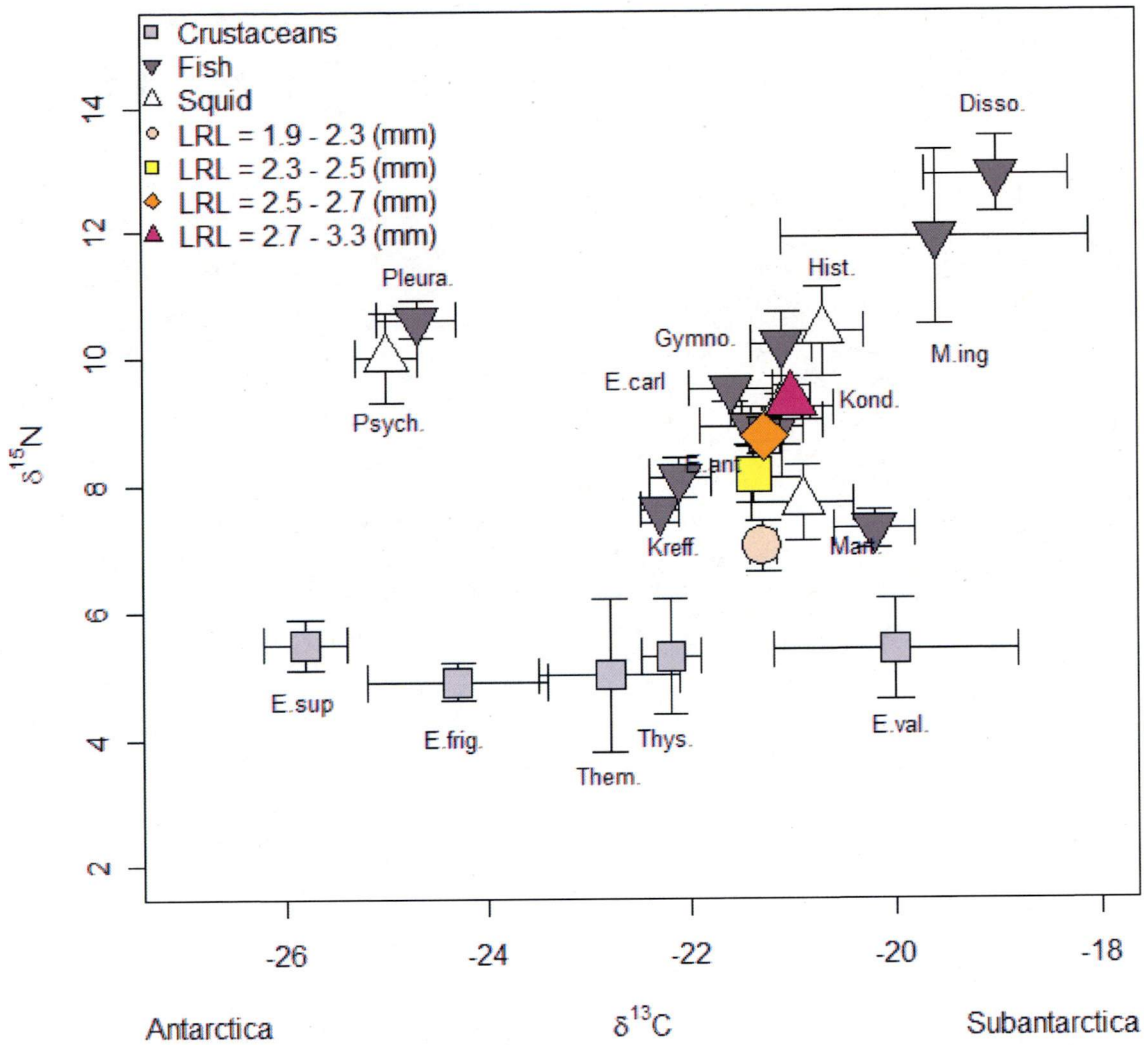

Figure 5 Comparing the mean and standard error of a corrected $\delta^{15}N$ (+ 3 ‰) and $\delta^{13}C$ values of *Histioteuthis eltaninae* grouped into four size classes (n = 71), with the mean and standard deviation of potential prey items.

3.1.4 Broad distribution of *Histioteuthis eltaninae*

No relationship occurred between the size of *Histioteuthis eltaninae* and $\delta^{13}C$ values ($r = 0.378$, $r^2 = 0.0175$, $F = 2.248$, $p = 0.1384$) (figure 6). $\delta^{13}C$ ranged from -22.41 ‰, with a mantle length of 69.19 mm to -19.8‰ corresponding to a mantle length of 74.69 mm. This confined carbon value aligns with the water mass that surrounds Macquarie Island and indicates that this species does not cross the Polar Front. There are also no seasonal effects on the carbon isotope values when grouping these values into months (figure 7).

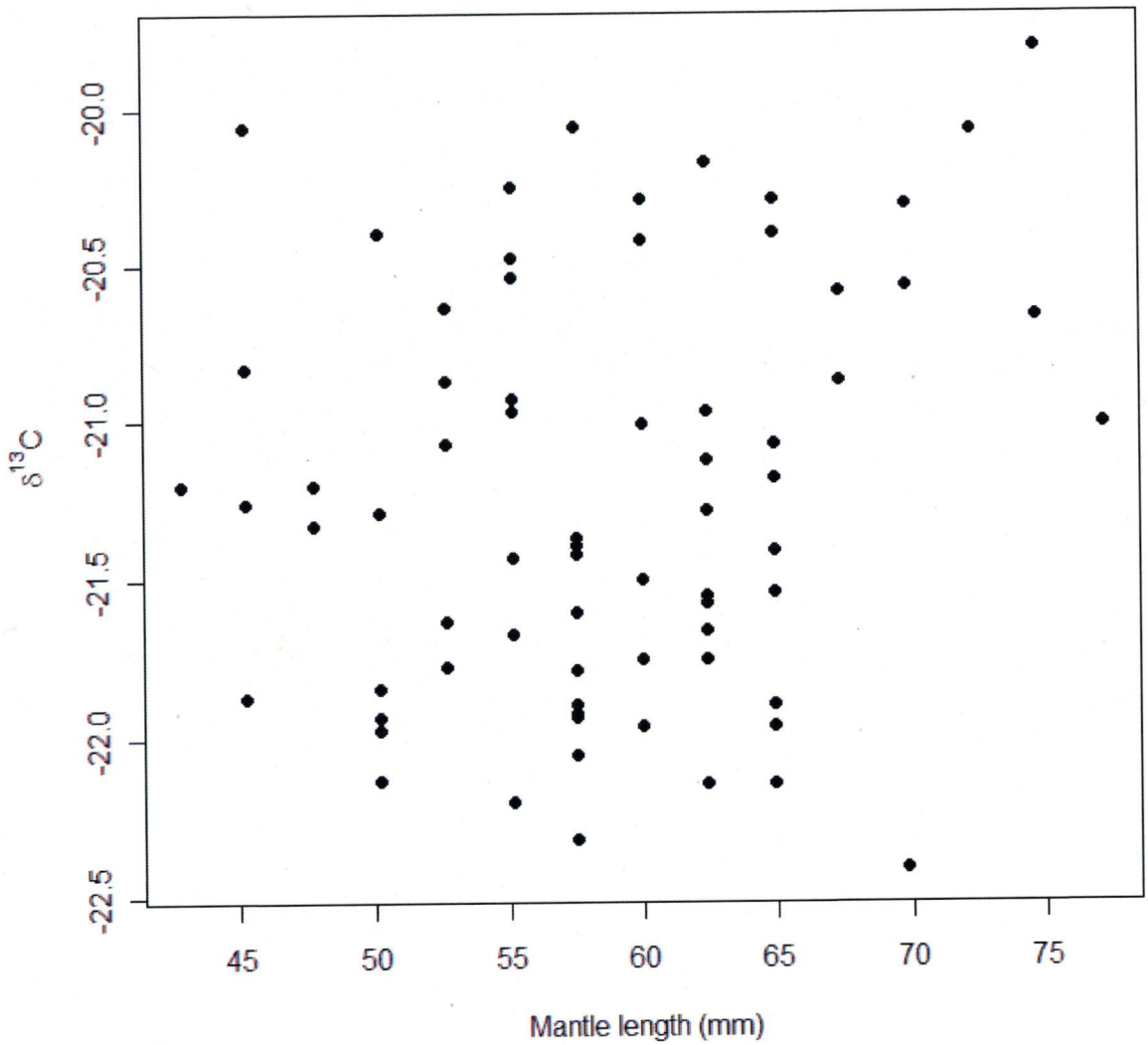

Figure 6 Relationship between $\delta^{13}C$ and calculated mass of *Histioteuthis eltaninae* (n = 71) represented by a scatter plot ($r = 0.378$, $r^2 = 0.0175$, $F = 2.248$, $p = 0.1384$).

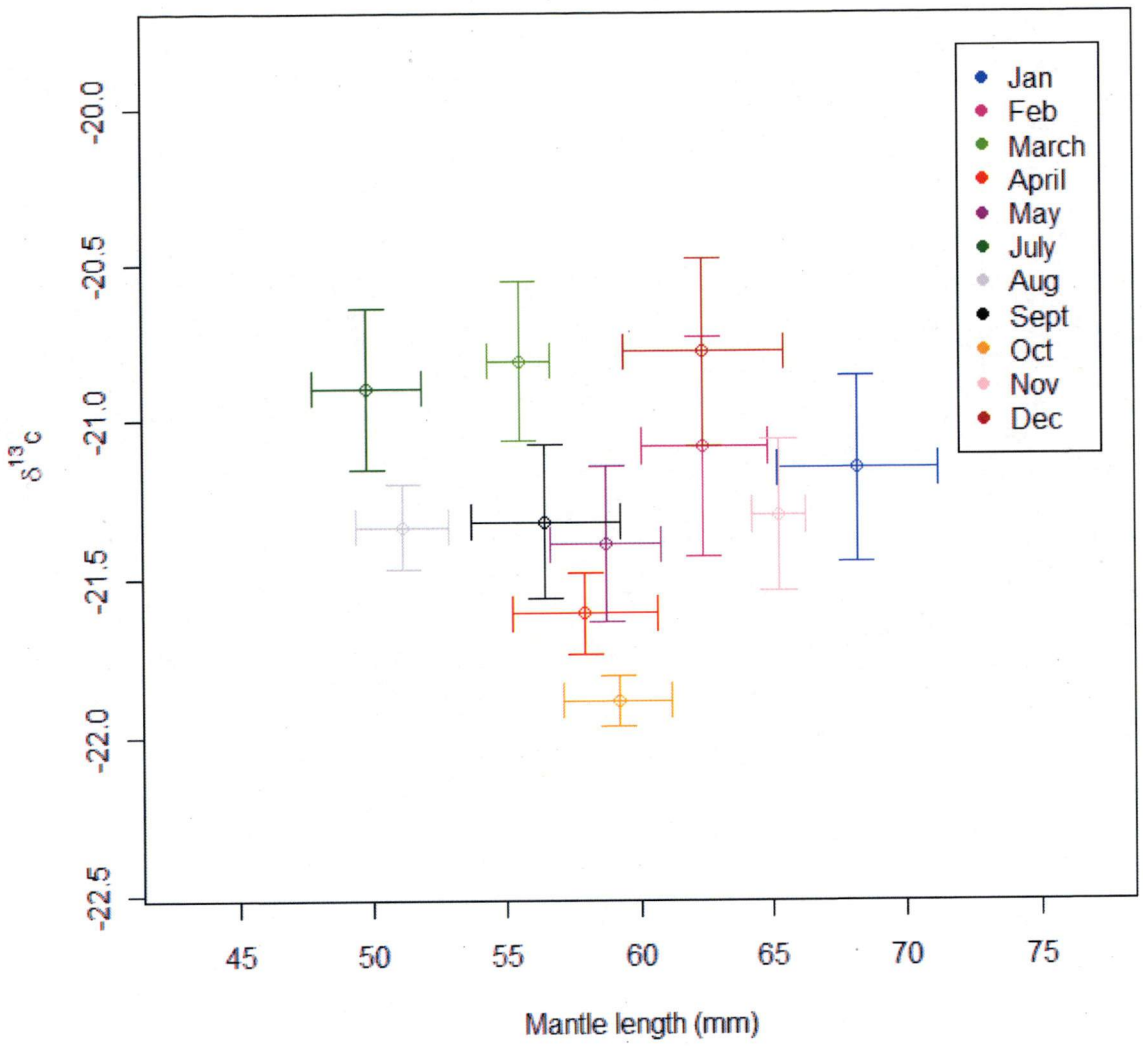

Figure 7 $\delta^{13}C$ values and the calculated mantle length of *Histioteuthis eltaninae* grouped by months (n = 71), with each month demonstrating the mean and standard error.

3.2 *Martialia hyadesi*

3.2.1 Size distribution of *Martialia hyadesi*

Martialia hyadesi is a much larger species than *Histioteuthis eltaninae*. All of the beaks retrieved from the seals (559 individual beaks), had a lower rostral length ranging from 1.36 mm to 13.01 mm. This corresponds to a mantle length of 142.08 mm to 485.41 mm respectively. The mean lower rostral length of these beaks was 7.41 mm that has a calculated mantle length of 320.28 mm.

There is variability in the size of all the beaks found in the stomach contents when grouped by month (figure 8). Generally there is an increase in calculated mean mantle length between February (297.58 mm) and June (333.63 mm) as well as between October and December, where there was an increase of almost 12 mm in mantle length. However, between June and August there is a mean decrease in mantle length from 333.63 mm to 320.95 mm respectively. October also decreases from the September mean mantle length by approximately 6.5 mm. In both January and February there is a decrease in the mean size when compared to the previous month, with decreases by 20.90 mm and 21.72 mm respectively in mantle length.

When considering the subset that was used for the stable isotopes analysis, their sizes ranged from 3.0 mm in lower rostral length to the largest of 11.6 mm lower rostral length. 66 individuals were randomly selected for this analysis, which is almost 12 % of the total *M. hyadesi* beaks found in the stomach contents. The mean size of the beaks used in this analysis was 7.94 mm in lower rostral length, which has a calculated mantle length of 335.96 mm.

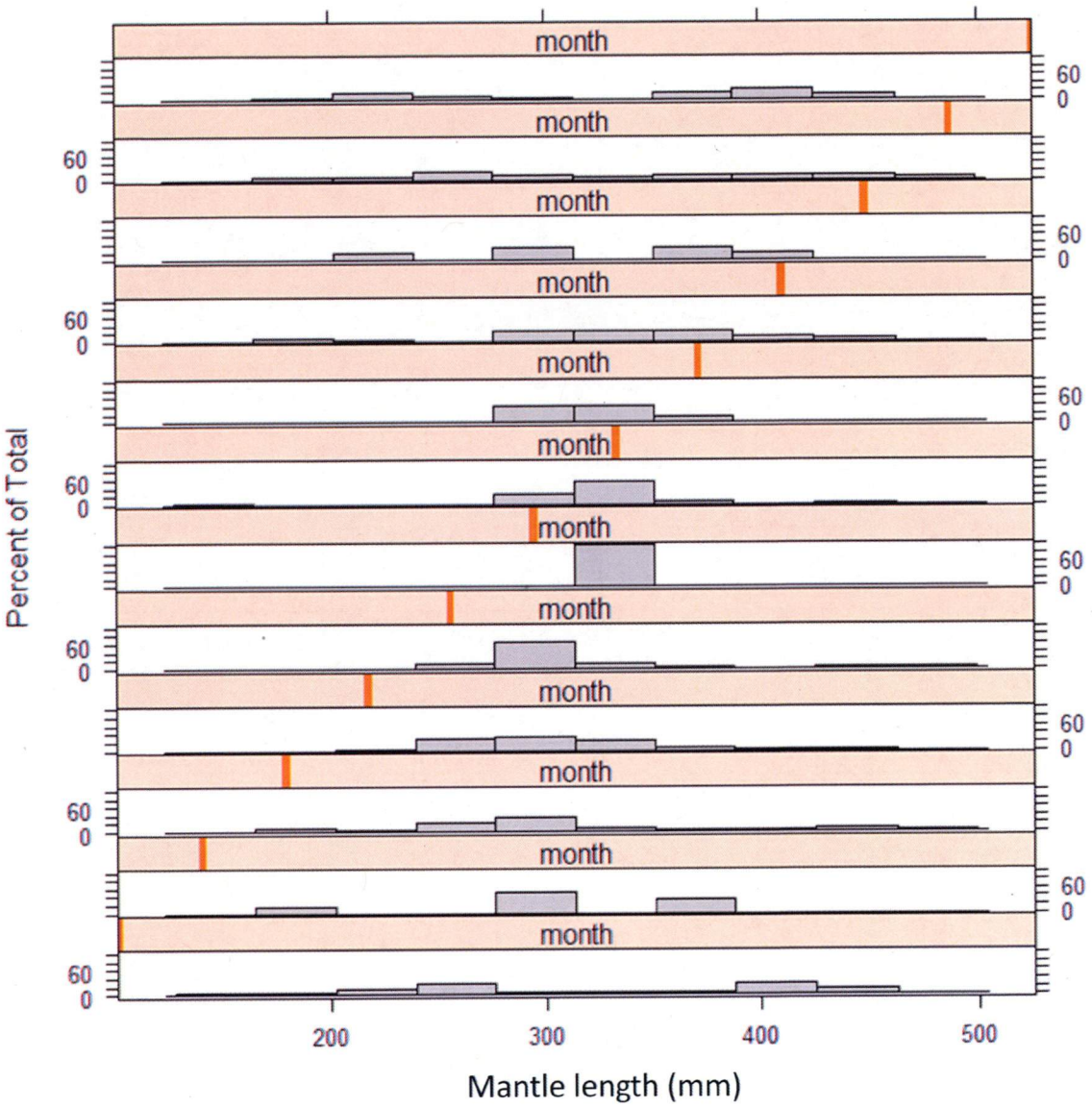

Figure 8 Size distribution (mantle length (mm)) of all *Martialia hyadesi* beaks found the stomach contents of southern elephant seals (n = 559) over a 12 consecutive month period.

3.2.2 Trophic position of *Martialia hyadesi*

The $\delta^{15}N$ values for *M. hyadesi* ranged from 8.16 ‰ (almost three trophic levels) which had a lower rostral length of 9 mm (mantle length of 367.23 mm) to the smallest value of -0.93 ‰ which had a lower rostral length of 5.6 mm (mantle length of 267.032 mm).

There were two individuals that demonstrated negative $\delta^{15}N$ values which were removed from the data set as it is not possible to have a negative trophic level. The positive linear relationship without these two negative nitrogen values remained significant (r = 0.5545,

$r^2 = 0.1696$, F = 11.83, p = < 0.05) (figure 9). The smallest positive nitrogen isotope value for this species was 0.9 ‰ that had a calculated mantle length of 293.56 mm from a lower rostral length of 6.5 mm.

The smallest individual subjected to stable isotope analysis, with an estimated mantle length of 190.41 mm (calculated from a lower rostral length of 3 mm), had a nitrogen value of 7.15 ‰. The largest individual, with a lower rostral length of 11.6 mm (resulting in a calculated mantle length of 443.852 mm) had a $\delta^{15}N$ value of 6.71 ‰

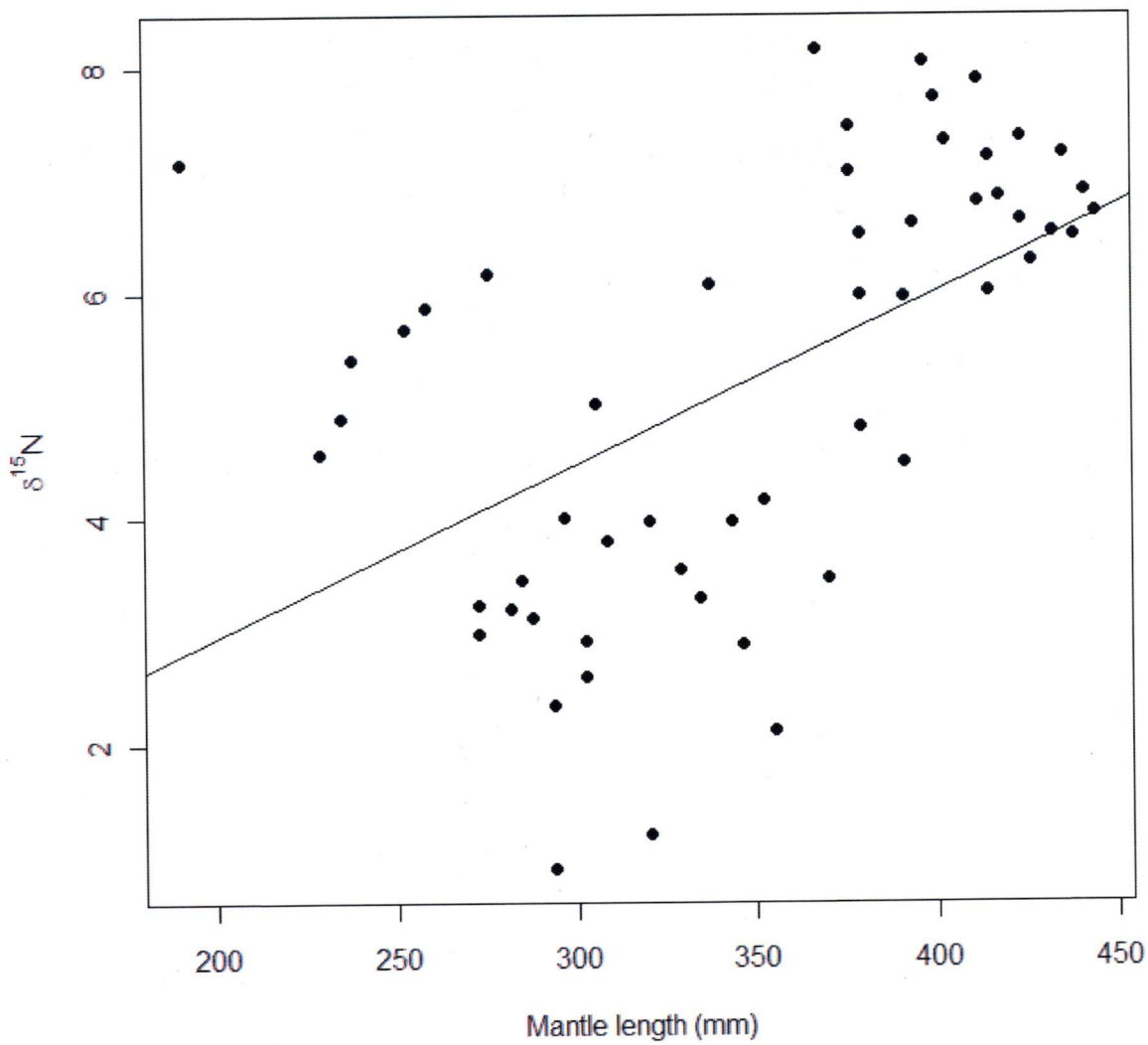

Figure 9 Relationship between $\delta^{15}N$ and calculated mantle length of *Martialia hyadesi*. Regression line: y = 0.016x - 0.4562 (r = 0.5545, $r^2 = 0.1696$, F = 11.83, p = < 0.05; n=64 (two $\delta^{15}N$ negative values were excluded).

This positive linear relationship between $\delta^{15}N$ and calculated mantle length does not appear to be strongly confounded by the effects of seasons (figure 10). However, February

and March had smaller individuals when compared to January and April. While these two months demonstrated a smaller mean mantle length of 306.82 and 258.68 mm respectively, they demonstrated a higher mean $\delta^{15}N$ value of 6.125 ‰ for February and 5.877 ‰ for March. This is approximately on par with the mean $\delta^{15}N$ value of January (6.774 ‰), but is approximately 2 to 2.5 ‰ higher than February (6.125 ‰) and March (5.877 ‰) respectively when compared to April ($\delta^{15}N$ value of 3.79 ‰).

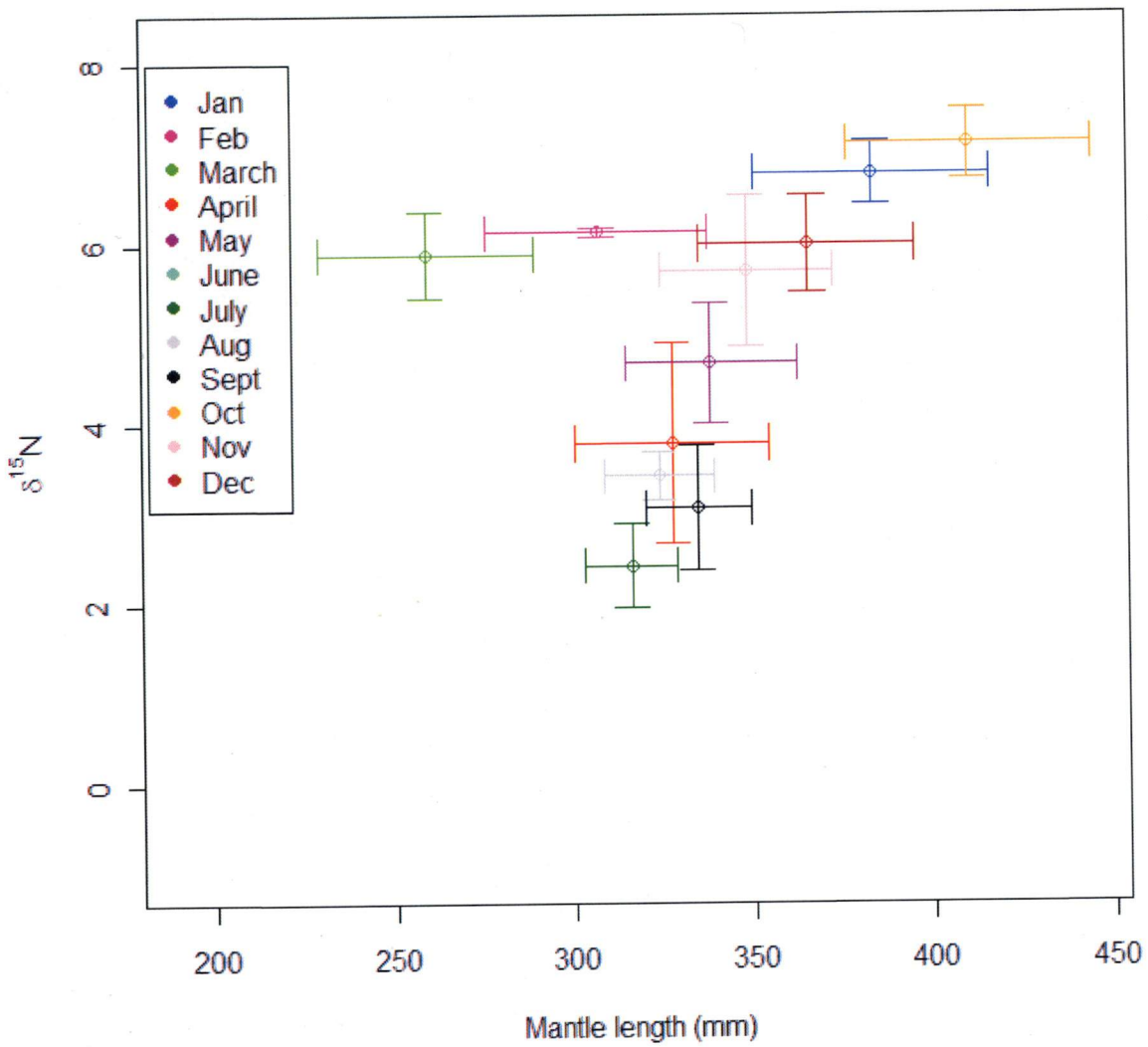

Figure 10 $\delta^{15}N$ and calculated mantle length of *Martialia hyadesi* grouped into months, demonstrating the mean and standard error (n = 64).

When comparing the nitrogen signatures that occurred between the two concurrent size classes between September and December, there was a significant difference between these two groups (*t*-test, t = 3.68, p = 0.001; n = 29) (figure 11.b). The beaks from the smaller group (lower rostral length < 7 mm) had a mean $\delta^{15}N$ value 3.46 ‰, equating to one

trophic level (n = 10). This smaller size class had a $\delta^{15}N$ range between 0.94 ‰, which had a lower rostral length of 6.4 mm to a $\delta^{15}N$ value of 5.8 ‰ which has a lower rostral length of 4.6 mm. The larger group (lower rostral length > 7 mm) had a mean $\delta^{15}N$ of 6.12 ‰ (n = 19), approximately twice the value of the smaller cohort. This approximately equates to two trophic levels, assuming that 3 ‰ is an accurate representation of a single trophic level increase for a cephalopod beak. The trend of smaller individuals feeding on lower trophic trophic levels is consistent in the overall trend occurring in all of the stable isotope analysis for *Martialia hyadesi*. This leads to the conclusion that the confounding effect of time of year is not as great an influence on the overall relationship between $\delta^{15}N$ and size.

Over this four month period there was a single negative $\delta^{15}N$ value of -0.8 ‰ for a beak that had a lower rostral length of 7.8 mm. This sample was excluded from the dataset due to the inability of having a negative trophic level. The next smallest $\delta^{15}N$ value over this time was 2.13 ‰, from a beak that had a lower rostral length of 8.6 mm.

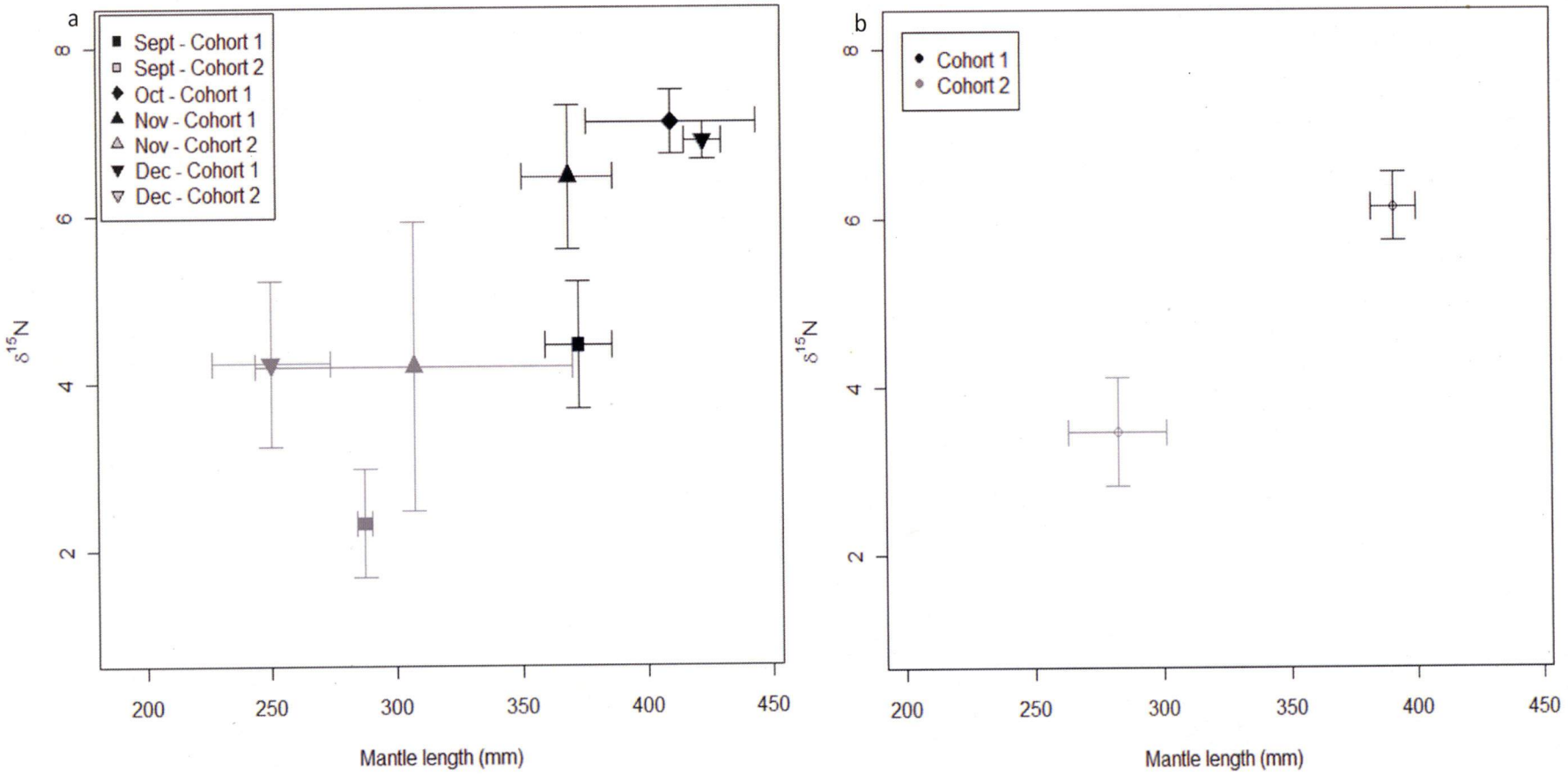

Figure 11 Relationship between mantle length and $\delta^{15}N$ for *Martialia hyadesi* between September and December demonstrated by the mean and standard error of the two concurrent size groups (cohort 2 lower rostral length < 7 mm, n=10; cohort 1 lower rostral length > 7 mm, n = 19). $\delta^{15}N$ values were significantly different between the two size groups (*t*-test, t = 3.68, p = 0.001).

a. Demonstrates the mean $\delta^{15}N$ for each cohort for each month.

b. Demonstrates the mean $\delta^{15}N$ value for the two cohorts over the four months

3.2.3 Potential prey of *Martialia hyades*

The squid *Martialia hyadesi* where grouped into four equal sampled size classes in order to compare against the stable isotope ratios of their potential prey resources. With a significant relationship occurring between the size of *M. hyadesi* and trophic level, the grouping of size classes allows for the examination of potential prey shifts with size. For these four size classes, the mean $\delta^{15}N$ and $\delta^{13}C$ values were determined (table 3). The mean $\delta^{13}C$ value ranged from -18.75 ‰ (in the largest size class) to -21.68 ‰ (the second smallest size class). The same trend occurred with the mean $\delta^{15}N$ values, where the smallest nitrogen signature occurring in the second smallest size class, with a corrected $\delta^{15}N$ value of 5.7 ‰ to the largest corrected nitrogen value of 9.99 ‰, which occurred in the largest size class.

Table 3 The mean stable isotope value for each of the size classes used to determine the potential prey of *Martialia hyadesi*. $\delta^{15}N$ needed to be corrected by an additional 3 ‰ in order to be compared to the nitrogen isotope values of other potential prey species.

Size classes (lower rostral length)	Mean of $\delta^{15}N$	Corrected mean of $\delta^{15}N$	Mean of $\delta^{13}C$
3 - 6.3 mm	4.05 ‰	7.05 ‰	-20.95 ‰
6.3 -7. 8 mm	2.70 ‰	5.70 ‰	-21.68 ‰
7.8 - 9.9 mm	5.19 ‰	8.19 ‰	-19.66 ‰
9.9 - 11.6 mm	6.99 ‰	9.99 ‰	-18.75 ‰

There is a large isotopic range of the four size classes of *M. hyadesi* in both carbon and nitrogen values. When comparing the size classes of potential prey items that have been found in the literature (table 1), there appears to be a clear separation between potential prey items and *M. hyadesi* in regards to $\delta^{13}C$ values (figure 12). Currently, there is a lack of stable isotope data on possible prey items in the regions that *M. hyadesi* largely foraged in, with this squid population foraging further north than the focus of the literature in regards to the stable isotope analysis of lower trophic levels.

When determining potential prey, there are several possible explanations for the isotope ratios that occur in the predator. The stable isotope value of the predator could correspond directly to possible prey item. However, it is also possible that the isotope signature in the predator is a mixture of higher and lower $\delta^{15}N$ values from their consumption. Squid

however, are only able to predate on individuals that are as large as themselves and this can therefore give greater indication of the best prey scenarios. In regards to *M. hyadesi,* the $\delta^{15}N$ values do appear to correspond with a broad array of possible prey items, including crustaceans, fish and other squid species.

When considering the smallest size classes, with a lower rostral length between 3 to 6.3 mm (corrected $\delta^{15}N$ value of 7.05 ‰ and $\delta^{13}C$ value of -20.95 ‰), these individuals align best with a myctophid species *Electrona subaspera*. This fish species has a $\delta^{15}N$ signature of 7.3 ‰ (approximately 0.25 ‰ more than *M. hyadesi*) and $\delta^{13}C$ -20.2 ‰ (0.75 ‰ difference to *M. hyadesi*). However, this species has the potential to prey on a mixture of higher and lower trophic levels than themselves in order to obtain their mean nitrogen isotope values. The maximum calculated mantle length for this size class is 287.66 mm. Therefore these individuals could potentially be predating on the myctophid fish, *Electrona antarctica* and *E. carlsbergi*, which have a maximum size of 85 mm and 97 mm respectively. In order to obtain the nitrogen value observed in this size group, they would also have to be predating upon smaller trophic level prey items such as the juvenile *Thysanoessa* species and *Euphausia vallentini.*

The next size class with a lower rostral length between 6.3 and 7.8 mm (with a corrected $\delta^{15}N$ value of 5.70 ‰ and $\delta^{13}C$ of -21.68 ‰) corresponds with two euphausiids crustaceans. More specifically, the juvenile *Thysanoessa* species, which differs in their $\delta^{15}N$ signature by approximately 0.4 ‰ and $\delta^{13}C$ by 0.52 ‰ to *M. hyadesi*. There is an association with *Euphausia vallentini* which has a $\delta^{13}C$ value of -20.0 ‰ (a difference of 1.68‰ with *M. hyadesi*) and $\delta^{15}N$ of 5.4‰ (difference of 0.3‰). When considering the possibility of this size class of *M. hyadesi* predating on a mixture of higher and lower trophic levels then the nitrogen value indicates, this appears to be unlikely with the smallest $\delta^{15}N$ signature of possible prey items being 5 ‰ in *Themisto gaudichaudii.*

The size classes that comprises of individuals with a lower rostral length between 7.8 to 9.9 mm has a corrected nitrogen stable isotope value of 8.19 ‰, and carbon isotope value of -19.66 ‰. This size class correspond best in regards to $\delta^{15}N$, with the myctophid *Protomyctophum tenisoni* (8.1 ‰), with a difference of 0.09 ‰. The $\delta^{13}C$ value differed by approximately 2.5 ‰, with the fish having a greater negative value of -22.1 ‰. It is also possible that the mean nitrogen value obtained for this size group is due to the predation of

Histeotheuthis eltaninae and *Gemnoscopelus nicholsi* in conjunction with lower trophic crustacean and/or myctophid fish.

The largest size class of *M. hyadesi* had a lower rostral length between 9.9 and 11.6 mm, which has a corrected mean nitrogen stable isotope value of 9.99 ‰, and carbon value of -18.75 ‰. The available potential prey species found in the literature do not have carbon values as high as this and therefore need to be more strongly influenced by the $\delta^{15}N$ value when comparing the two isotope elements. Considering this factor, the most likely potential prey for this size group is the myctophid, *Gymnoscopelus nicholsi,* and the squid *Histioteuthis eltaninae*. The myctophid, *Gymnoscopelus nicholsi* has a $\delta^{15}N$ value of 10.2 ‰ and a $\delta^{13}C$ of -21.1 ‰, which differs by 0.31 ‰ and 2.35 ‰ respectively to *M. hyadesi*. In regards to *H. eltaninae*, there is a difference of 0.5 ‰ for the $\delta^{15}N$ value, with squid *H. eltaninae* having a value of -20.7 ‰. The difference between the $\delta^{13}C$ values of these two squid is almost by 2 ‰, with *H. eltaninae* value being -20.7 ‰. It is also possible that this size class is predating on a mixture of prey items at a higher and lower trophic level to obtain their isotope signatures. This size class has a maximum mantle length of 485 mm, and considering that squid are unable to predate on specimens larger than themselves, it is unlikely that this size classes is feeding on the Patagonian toothfish, which can obtain sizes over two meters. It is possible however, that this size class is predating on the ice fish, *Channichthys rhinoceratus,* which has a maximum length of 520 mm. If this is true then this size class would also be predating on lower trophic levels such as the myctophid fish *Protomyctophum tenisoni.*

However, despite the several possible scenarios of potential prey species for *M. hyadesi*, it appears that a diverse array of species is being predated on. Their prey appears to include crustaceans, fish, predominately myctophids, and other squid species. A shift in prey items also appears to be occurring, with smaller and therefore younger individuals feeding on a greater amount of crustacean species. As the population gets larger and therefore matures, they are feeding on higher trophic levels, such as fish and other squid species.

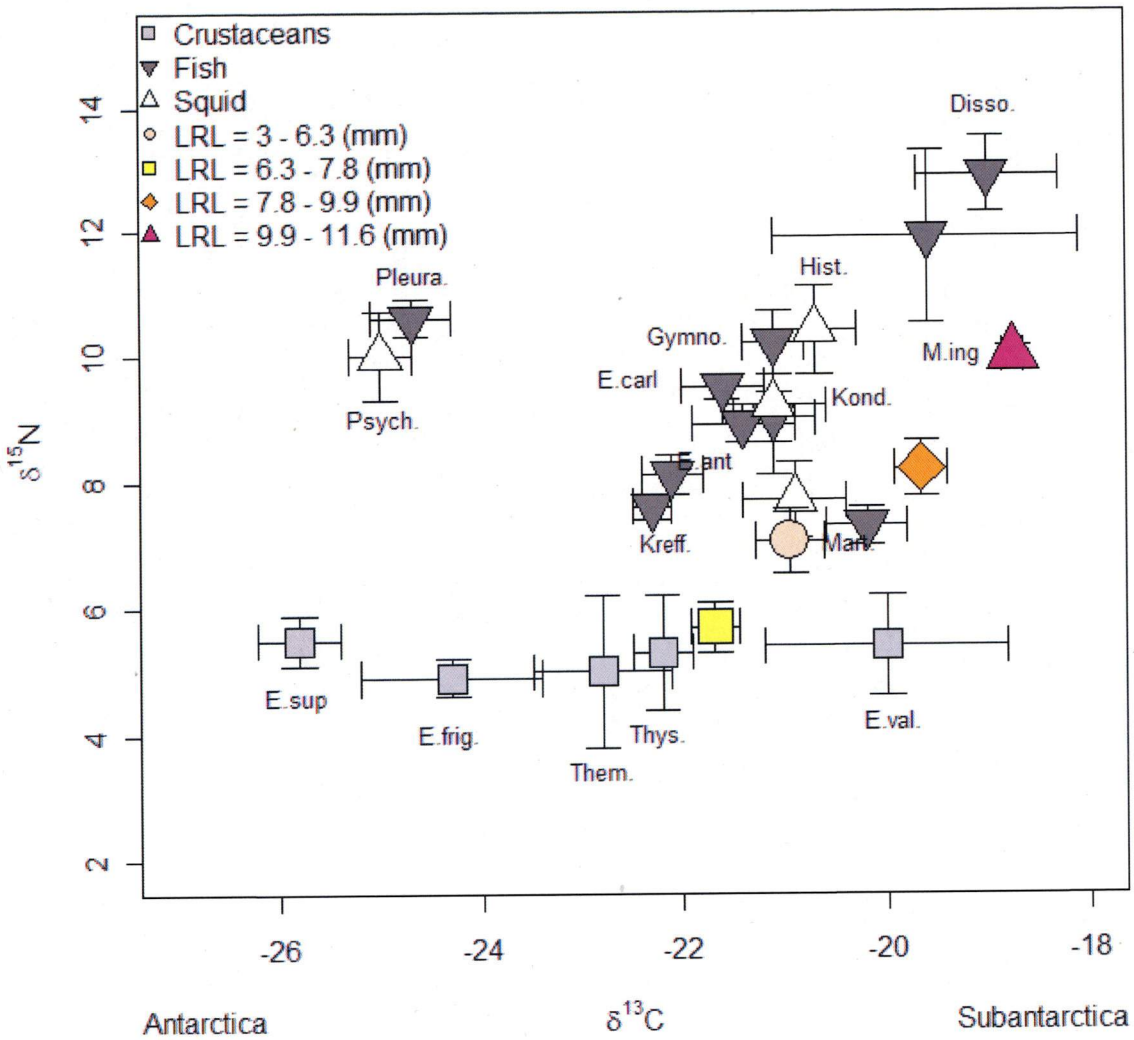

Figure 12 Comparing the mean and standard error of a corrected $\delta^{15}N$ (+ 3‰) and $\delta^{13}C$ values of *Martialia hyadesi* grouped into four size classes (n = 66), with the mean and standard deviation of potential prey items.

3.2.4 Broad distribution of *Martialia hyadesi*

There is a significant linear relationship occurring between the $\delta^{13}C$ values and the size of *Martialia hyadesi* (figure 13) ($r = 0.5719$, $r^2 = 0.2324$ $F = 20.07$, $p = < 0.05$). The carbon values for all the sampled individuals of *M. hyadesi* ranged between -23.89 ‰ and -17.73 ‰, which is a range of 6.16 ‰. These carbon isotope values correspond with a distribution between the southern boundary of the Antarctic Circumpolar Current to northern waters between Macquarie Island and southern Australia. The individual that had a distribution that were southernmost, as indicated by the $\delta^{13}C$ value of -23.89 ‰, had a

calculated mantle length of 290.61 mm. The northernmost *M. hyadesi* individual (-17.73 ‰) had a calculated mantle length of 414.48 mm

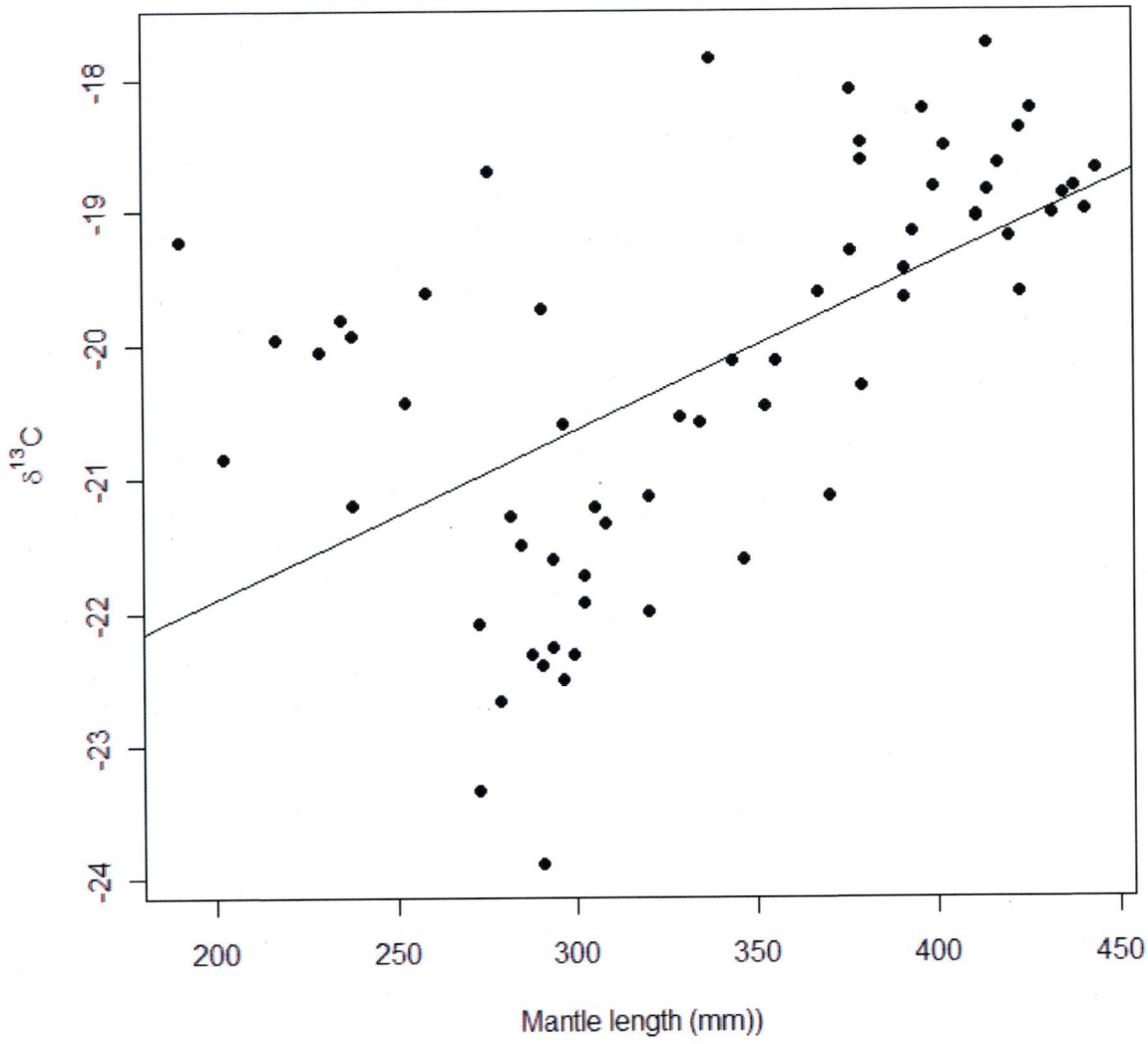

Figure 13 Relationship between $\delta^{13}C$ and calculated mantle length (mm) of *Martialia hyadesi*. Regression equation line: y = 0.0126x - 24.436 (r = 0.5719, r^2 = 0.2324 F = 20.07, p = < 0.05; n=64, with the two negative values excluded).

This positive linear relationship between $\delta^{13}C$ and calculated mantle length does not appear to be strongly confounded by the effects of seasons (figure 14). However, February and March had smaller individuals when compared to January and April. These two smaller mean months (February and March) had a higher $\delta^{13}C$ value of -18.27 ‰ and -19.74 ‰ respectively. This is approximately on par with the mean $\delta^{13}C$ value of January (-19.11 ‰), but is approximately 2 ‰ to 0.5 ‰ higher than February (-18.27 ‰) and March (19.74 ‰) respectively when compared to April ($\delta^{13}C$ value of -20.28 ‰). However, all of these

carbon values indicate a distribution that is around the Macquarie Island region, with them all having a distribution that are north of the Polar Front.

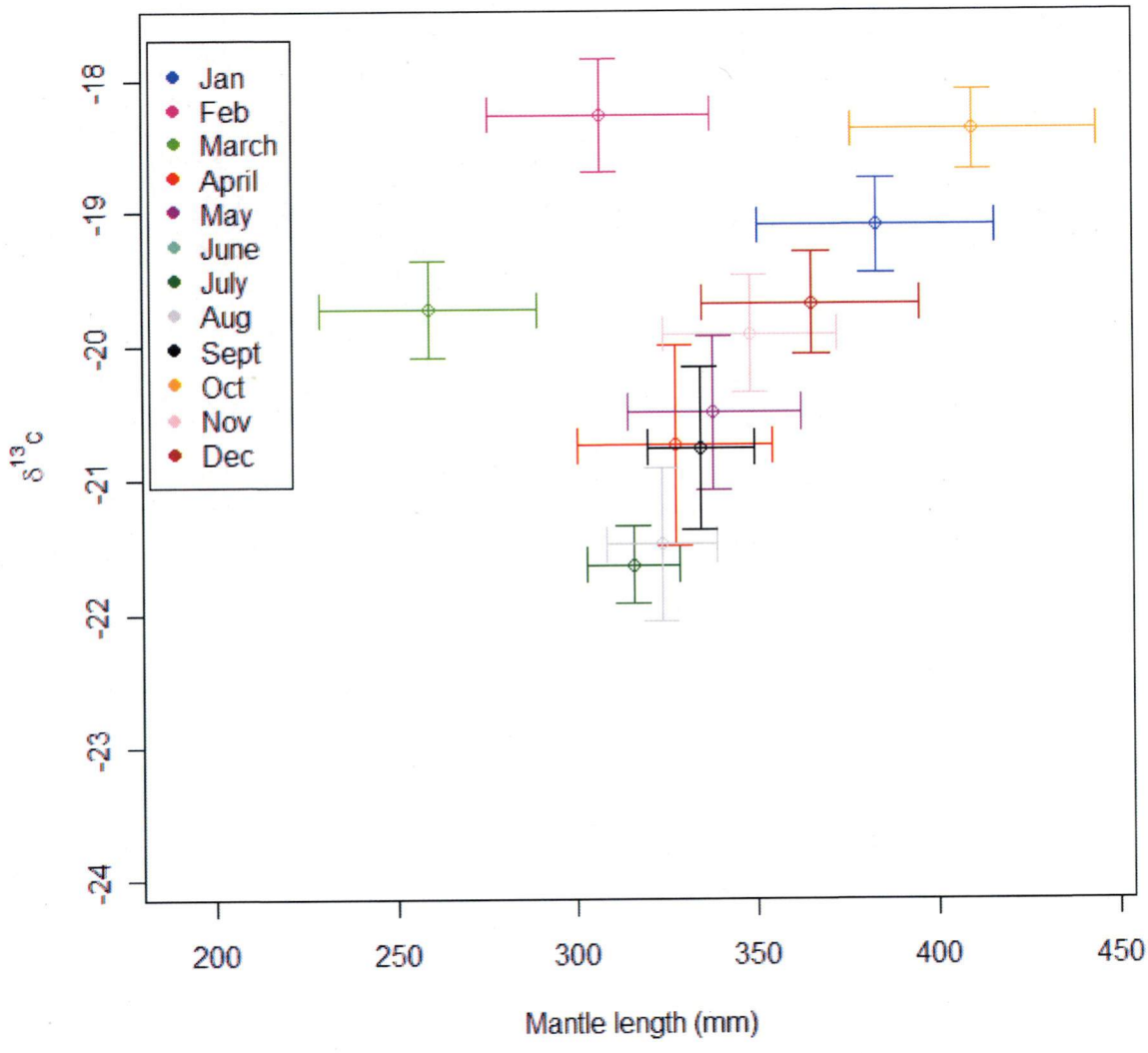

Figure 14 Relationship between $\delta^{13}C$ values and calculated mantle length of *Martialia hyadesi* that have been grouped into months, demonstrating by mean and standard error (n = 64).

Between September and December, two concurrent size cohorts occurred. The smaller size class contained individuals that had a lower rostral length that was less than 7 mm (n = 10). This size class was significant different in their $\delta^{13}C$ signatures (*t*-test, t = 4.56, p = < 0.05) than the larger sized cohort that had individuals with a lower rostral length over 7 mm (to 11.6 mm; n = 19) (figure 15). The smaller size cohort had a mean $\delta^{13}C$ signature of -21.34 ‰, with the larger group demonstrating an increase in approximately 2 ‰, with a

mean of -19.25 ‰. The lower carbon value of the smaller individuals represents a higher latitude, which corresponds to a general distribution in southern Macquarie Island waters. This indicates that the individuals in the smaller cohort occur further south to almost the Polar Front, when compared to the larger individuals during the same four month period, which occur in waters that are located north of the island.

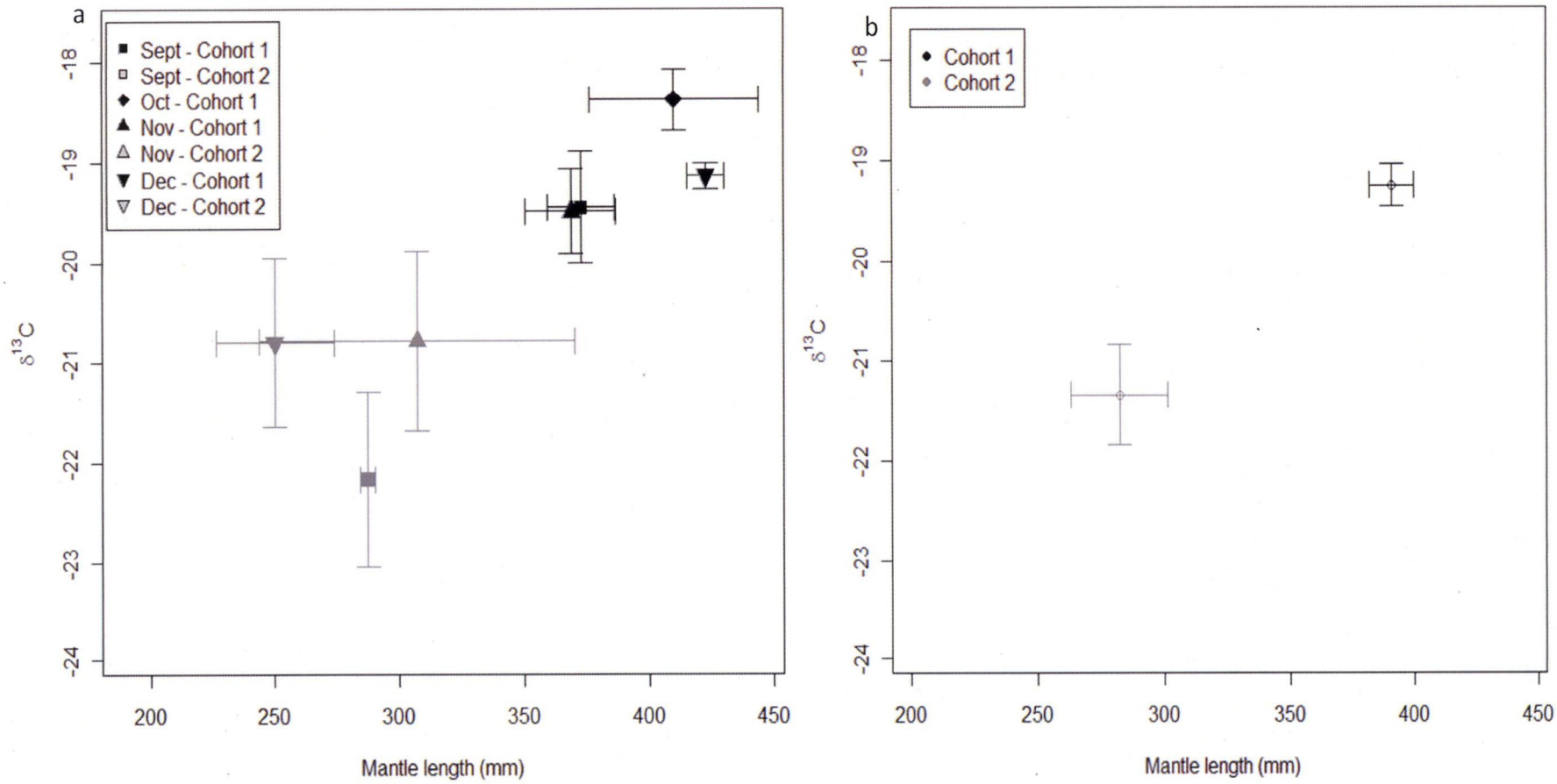

Figure 15 Relationship and significant difference between the 2 concurrent size cohorts (cohort 2 < 7 mm lower rostral length; n = 10 and cohort 1 > 7 mm lower rostral length; n = 19) in regards to $\delta^{13}C$ values for *Martialia hyadesi* (*t*-test, t = 4.56, p= < 0.05; n = 29). The data has been separated by month and/or cohort and is represented by the mean and standard error.

a. Demonstrates the mean $\delta^{13}C$ for each cohort in each month.

b. Demonstrates the mean $\delta^{13}C$ value both cohorts over the four month period.

3.3 Comparison of *Histioteuthis eltaninae* and *Martialia hyadesi*

3.3.1 Comparison of trophic level of *Histioteuthis eltaninae* and *Martialia hyadesi*

When comparing the nitrogen stable isotope values of *Histioteuthis eltaninae* and *Martialia hyadesi*, there is no significant difference between the two species in regards to their trophic level (*t*-test, t=1.11, p= 0.27; n=135). *H. eltaninae* has a slightly higher mean by 0.36 ‰, when compared to *M. hyadesi*, with a mean $\delta^{15}N$ value of 5.29 ‰ and 4.93 ‰ respectively (figure 16).

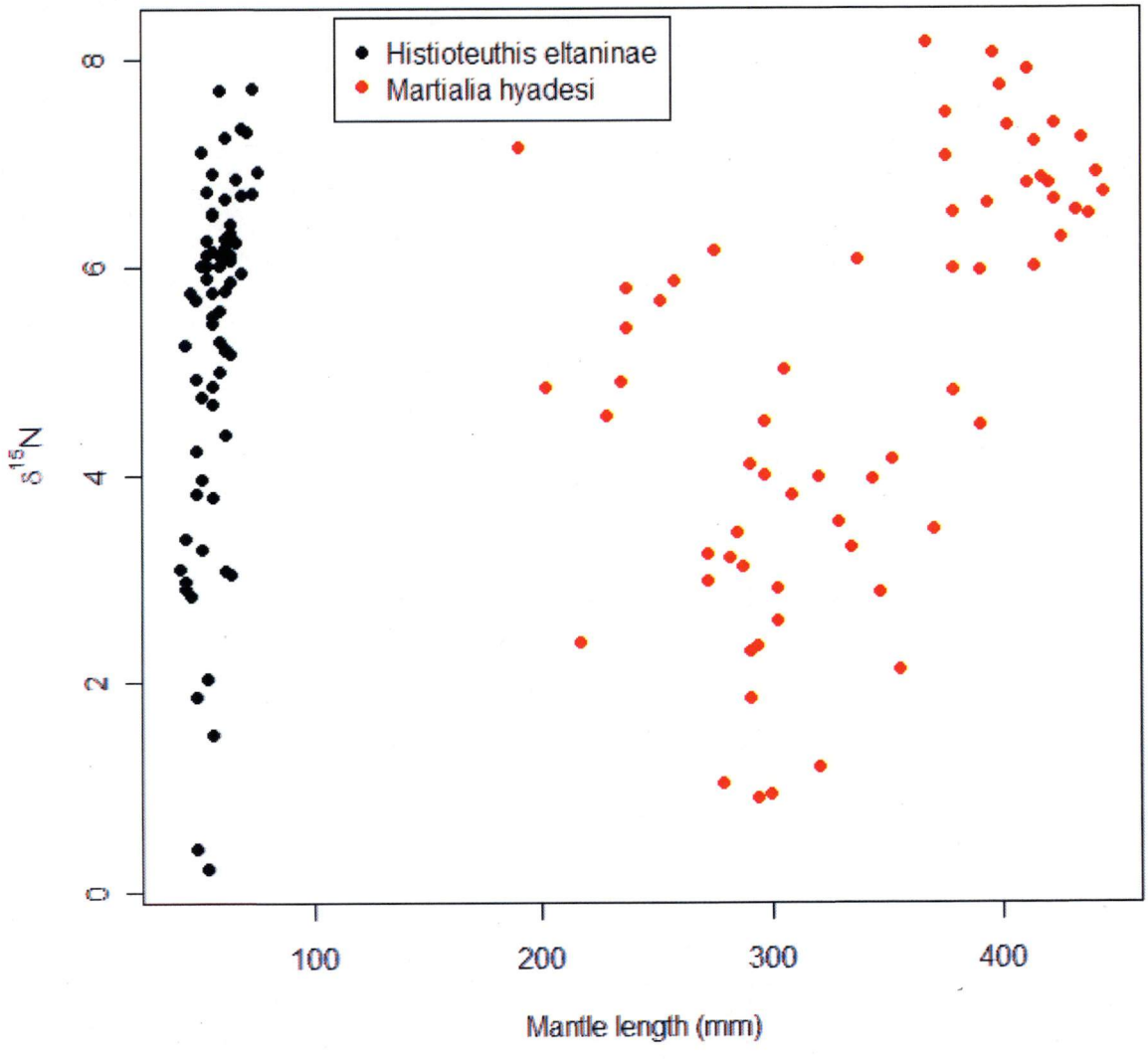

Figure 16 Comparison between *Histioteuthis eltaninae* and *Martialia hyadesi* in regards to their relationship between $\delta^{15}N$ and size (*t*-test, t = 1.11, p = 0.27; n =135).

3.3.2 Comparison of broad distribution of *Histioteuthis eltaninae* and *Martialia hyadesi*

When comparing *Histioteuthis eltaninae* (n = 71) and *Martialia hyadesi* (n = 64), there is a significant difference in their distribution, represented by the $\delta^{13}C$ signatures (*t*-test, t = -5.47, p = < 0.05; n =135). *H. eltaninae* has a more restricted distribution indicated by the carbon isotope signature, with a range of 2.61 ‰, which ranged from -22.24 ‰ to -20.18 ‰ and with a mean of -21.24 ‰, This indicates a distribution that is relatively confined to the waters around Macquarie Island. This is compared to *M. hyadesi* that has more than twice the range as indicated by the $\delta^{13}C$ value (6.16 ‰). This greater diversity of distribution occurs both to the north and south, by 2.7 ‰ and 1.48 ‰ respectively (figure 17). This indicates that *M. hyadesi* has a distribution that ranges from the southern borders of the Antarctic Circumpolar Current to waters that extend further north of Macquarie Island, but not quite to Australian waters. The mean for *M. hyadesi* is located further south than *H. eltaninae*, by approximately 1‰ (-20.25 ‰), but both means are located north of the Polar Front.

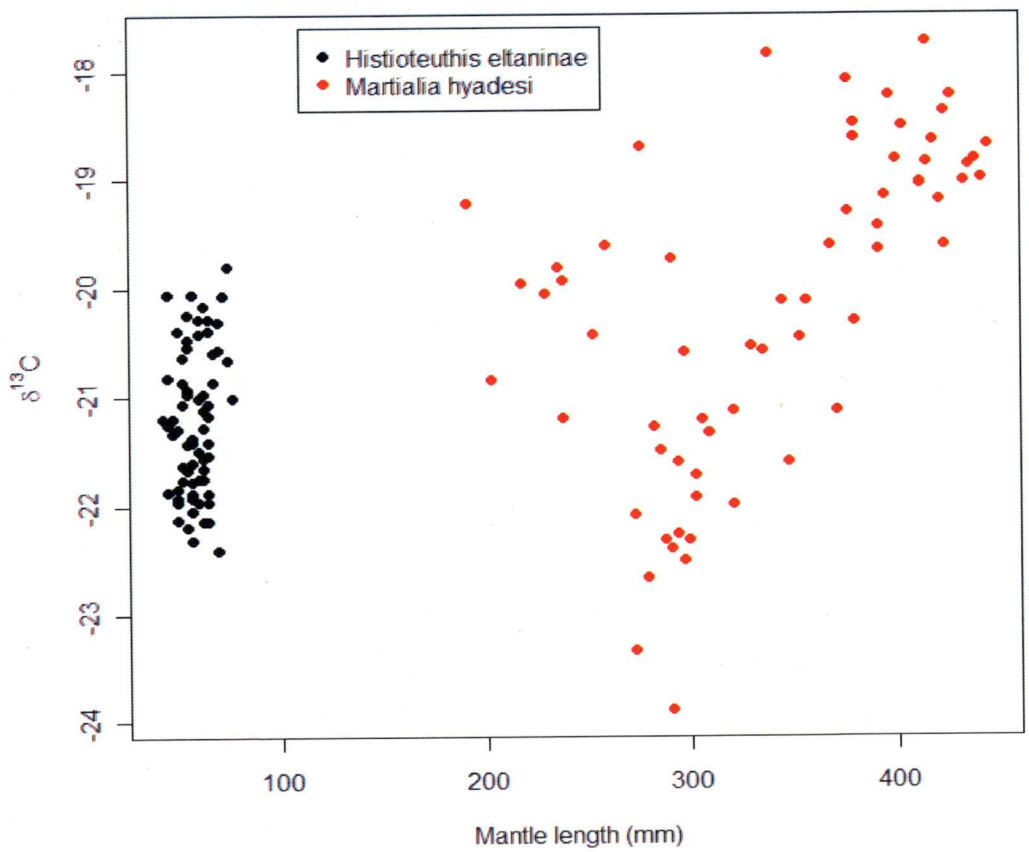

Figure 17 Comparison and significant difference between *Histioteuthis eltaninae* and *Martialia hyadesi* in regards to their relationship between $\delta^{13}C$ and size (*t*-test, t =-5.47, p = < 0.05; n=135).

3.3.3 – Overall comparison of stable isotope analysis of *Histioteuthis eltaninae* and *Martialia hyadesi*

When looking at the nitrogen and carbon isotope values for each of the species, *Martialia hyadesi* demonstrate a positive linear relationship between the two values, where *Histioteuthis eltaninae* showed no relationship between the two elements (figure 18). However, *M. hyadesi* demonstrate two clear peaks around the first and second trophic level (3 ‰ and 6 ‰ respectively). When comparing this to *H. eltaninae*, this species demonstrates one clear peak at approximately the second trophic level (6 ‰).

The $\delta^{13}C$ value for *H. eltaninae* ranges from -22.5 ‰ to -19.5 ‰ and demonstrates a gradual decrease in frequency, with the exception of a peak around -22.5 ‰. In regards to $\delta^{15}N$, the values range from 0 ‰ to 8 ‰. There is a clear peak around 6 ‰ with the highest frequency occurring at this second trophic level. There is then a decrease to approximately 4 ‰ and then increases slightly around the one trophic step level (3 ‰).

When looking at the range in the frequency plots for $\delta^{13}C$, *M. hyadesi* carbon values ranges between -24 ‰ to -17.5 ‰. There is a clear bimodal pattern occurring, with two peaks occurring around -22 ‰ and -19 ‰, the latter having the highest frequency. This same bimodal pattern occurs in the $\delta^{15}N$ stable isotope values, which ranges from negative numbers to 8.5 ‰. These two peaks occur around 6.5 ‰ and 3 ‰, which is approximately two and one trophic levels respectively.

When considering the predominate foraging regions of these two species, *H. eltaninae* is relatively confined to the second trophic level, which appear to be predated on over a narrow range around Macquarie Island. In contrast, *M. hyadesi* have a broader distribution in which to forage and on a greater diversity of prey items at the first and second trophic step. These predominate foraging regions do not appear to overlap between the two species, resulting in limited competition between the predominate resources that are utilized by *H. eltaninae* and *M. hyadesi* (figure 18).

A general linear model compared these two species and found that there was a significant difference between both the $\delta^{13}C$ (t-value = 7.318, $p = < 0.05$) and $\delta^{15}N$ values (t-value = -5.06, $p = < 0.05$) but found no month or season effect (t-value = 0.473, $p = 0.473$). The general linear model can therefore determine the species predominately from the isotope values and not from month. The general linear model determined that there was an overall significant difference between these two species in regards of the

stable isotope results and the effect of season ($r = 0.4225$, $r^2 = 0.2902$, $F = 19.53$, $p = < 0.05$).

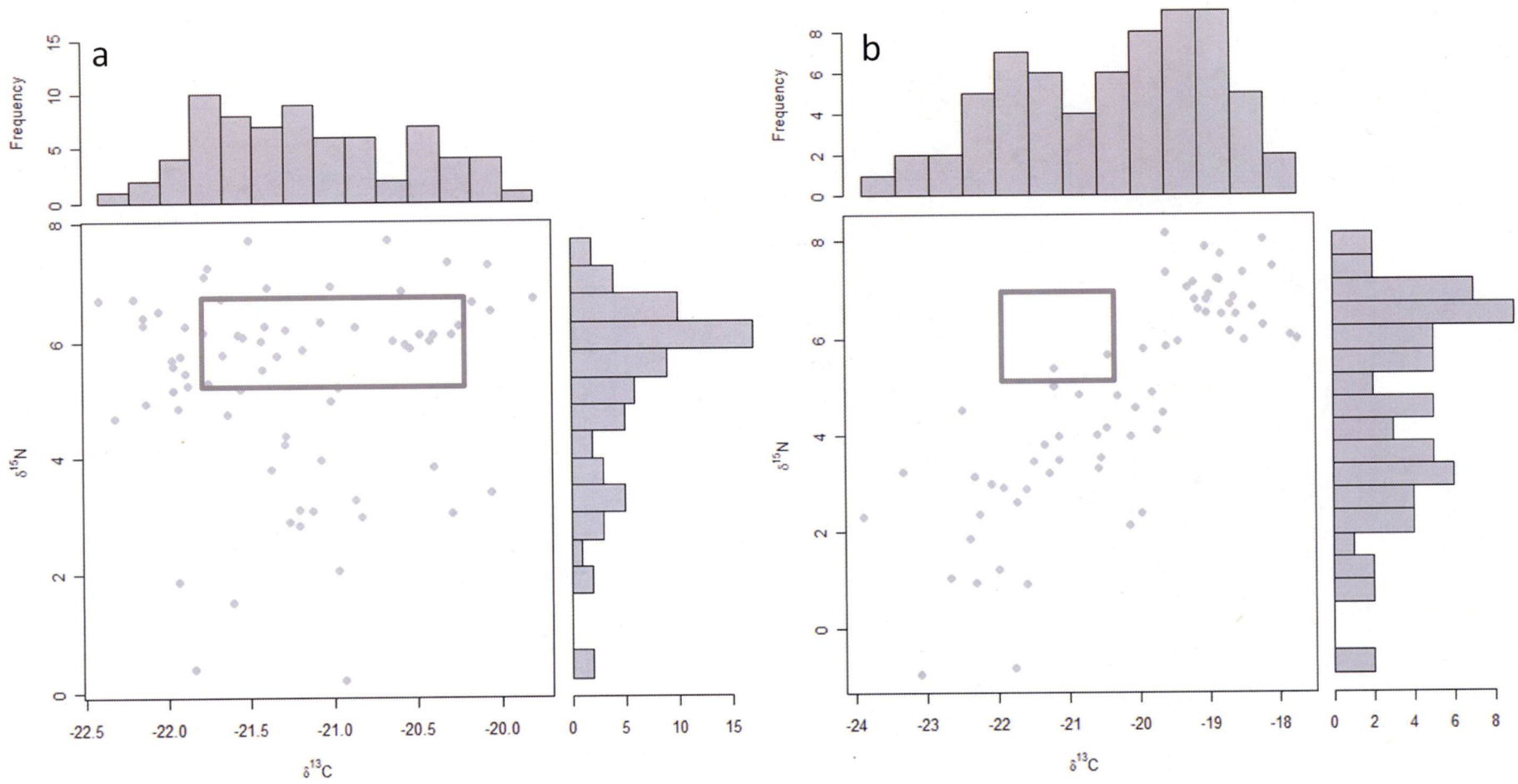

Figure 18 A comparison between **a.** *Histioteuthis eltaninae* and **b.** *Martialia hyadesi* in regards to $\delta^{13}C$ and $\delta^{15}N$ represented by a scatter plot and a frequency histogram. The box in both **a.** and **b.** graphs demonstrates where *Histioteuthis eltaninae* is predominately utilizing in regards to their distribution and prey resources.

4. DISCUSSION

There is a lack of information on cephalopods in the Southern Ocean largely due to the inability to catch specimens, particularly in the pelagic habitat (Clarke 1996). This is despite the use of several different kinds of nets of various different sizes, which can which can catch hundreds of fish in one haul in, but usually catches less than eight cephalopods. (Clarke 1996).

Therefore, alternative methods of sampling are required. One method which has considerable promise is utilizing cephalopod predators to sample these populations instead (Cherel and Weimerskirch 1995). Cephalopods are key prey items and consumed in large proportions by apex predators such as seals, seabirds, and whales (Stowasser *et al.* 2006). Cephalopods have resistant, indigestible mandibles (also known as beaks) which accumulate in the stomachs of predators (Lu and Ickeringill 2002). The beaks have species specific morphology, so the analysis of stomach contents can yield considerable information on the predated populations of squid (Xavier and Cherel 2009). In addition, beaks can yield information on size (Rodhouse 1990), age (Oosthuizen 2004), and further analysis through stable isotope analysis can yield knowledge on trophic position, potential prey items and broad distribution (Ruiz-Cooley *et al.* 2006).

With the potential of exploitation of cephalopod species in Southern Ocean waters, particularly for *Martialia hyadesi,* with exploratory fishing trails already occurring, it is important to gain as much knowledge as possible into the population's biological parameters. As the typical cephalopods life cycle is short lived with fast growth and semelparous breeding, this group has a greater susceptibility to overexploitation (Jackson *et al.* 2000). In the Southern Ocean, an ill managed cephalopod fishery could have wide reaching and server consequences not only on the exploited population, but also on the higher trophic levels that rely on these individuals as a food source, but also the prey items which they consume themselves.

4.1 *Histioteuthis eltaninae*

Histioteuthid squids are a mesopelagic family which inhabit all of the world's oceans from the Sub-Arctic to the Sub-Antarctic waters (Laptikhovsky 2001). However, there is limited information on *Histioteuthis eltaninae*. The current knowledge is relatively restricted to the role this species plays as a resource for numerous higher order predators in the Antarctica and sub-Antarctic. For example, *H. eltaninae* lower beaks have been collected from Patagonian toothfish (*Dissostichus eleginoides*) and southern elephant seals (van den Hoff 2001). This is the first study investigating the distribution, trophic position and potential prey resources for *H. eltaninae* through using stable isotope analysis.

4.1.1 Size distribution of *Histioteuthis eltaninae*

Histioteuthis eltaninae that where consumed by the elephant seals at Macquarie Island increased in size from July (mantle length of 19.36 mm) to May (mantle length of 92.80 mm). These mantle length were larger than beaks recovered from previous studies on elephant seals and Patagonian toothfish by van den Hoff (2004), which had a range of calculated mantle length of 31.36 mm to 89.13 mm and 53.39 mm to 57.80 mm respectively. With there being no larger specimens captured in previous studies, this indicates that the stomach contents of this present study were an accurate representation of the size distribution of this population of *H. eltaninae*.

While there is a natural progression in size of the data over the 11 months period between July and May, it is difficult to speculate about the growth of this population. There is the potential that the seals bias the size distribution of the squid sampled by actively selecting certain size classes, resulting in an inaccurate representation of the population. However, this appears to be an unlikely influence with limited skewedness in the size distribution of this species from the stomach contents of the elephant seals (figure 2). That the seals demonstrate a more opportunistic predation strategy that does not display prey size selection (McConnell *et al.* 1992). Therefore, it is likely that the elephant seals provided a near random selection of the available squid.

It is also difficult to estimate the growth of a squid due to their characteristic life histories. They have a short life span, usually ranging from a few months to two years that demonstrates fast and variable growth during this time (Rodhouse and White 1995). Their

growth is also strongly influenced by biotic and abotic factors, predominately water temperature and food availability (Oosthuizen 2004). With their short life span, this results in environmental factors causing different growth rates between each of the cohorts. This therefore makes it difficult and unreliable to presume and estimated age from length. It would result in the overestimation of longevity and an underestimation of the growth rates during their life span. Therefore the use of length-frequencies to estimate age and therefore growth, has been a questioned technique to apply to cephalopods since the 1980s (Jackson *et al.* 1997).

While growth is difficult to speculate about due to the inadequacies of length-frequencies, it does appear that this species has a life span of approximately one year. It is likely that smaller size classes are missing from this study due to the inability of elephant seals to predate on such small squid. While July contained the smallest individuals consumed by the elephant seals, this does not necessarily indicate that recruitment of *H. eltaninae* population occurs at this time. However, it is likely to occur just prior to this mid-year period.

4.1.2 Trophic position of *Histioteuthis eltaninae*

In the stable isotope analysis, smaller individuals were not utilized due to these beaks exhibiting clear wings which had not been chitinized (Cherel *et al.* 2009). The higher levels of chitin in these clear wings result in a limited $\delta^{15}N$ signature when compared to darkened wings (Hobson and Cherel 2006). Therefore clear and darkened wings cannot be compared without being confounded by this process (Hobson and Cherel 2006). This has resulted in the stable isotope analysis not being able to be conducted on individuals that had a lower rostral length smaller than 1.9 mm, almost 1 mm larger than the smallest identified *H. eltaninae* beak.

There was a positive linear relationship occurring between the $\delta^{15}N$ values and the size of the *H. eltaninae* individuals that the stable isotope analysis could be conducted on. The smaller individuals collected between July and September had a $\delta^{15}N$ ranged between 2.17 ‰ to 4.1 ‰ respectively, representing approximately one trophic level (3 ‰ (Cherel *et al.* 2009)). There is an increase of approximately two trophic levels (6 ‰) between November and May, which ranges from 6.26 ‰ to 5.6 ‰ respectively. This demonstrates

that as individuals get larger, they predate on higher trophic levels, utilizing a larger diversity of resources.

However, there is a seasonal effect occurring on this relationship between $\delta^{15}N$ values and size. This is to be expected though, with a species having an approximate longevity of one year resulting in a seasonal life history. Consequently, it is relatively undetermined if season is having a true confounding effect on the relationship between size and the $\delta^{15}N$ values.

4.1.3 Potential prey of *Histioteuthis eltaninae*

There are limited stable isotope data of potential prey species resources for cephalopod populations at Macquarie Island. However, for *Histioteuthis eltaninae*, there does appear to be a relatively good overlap between the data available on myctophids that are distributed around the Kerguelen region as well as a squid species, *Kondakovia longimana*.

There has only been one study that has investigated the stomach contents of *H. eltaninae* which examined two specimens that were caught in a pelagic trawl (RMT25) at the Antarctic Polar Frontal Zone in February 1994 (Rodhouse *et al.* 1996). These two individuals had a mantle length of 67 and 79 mm, which corresponds to the largest size class in the current study (Rodhouse *et al.* 1996). The stomach contents of these two individual included myctophid fish, crustaceans as well as another cephalopod. The stable isotope ratios of the larger individuals greater than 58 mm in mantle length in the current study were comparable to myctophids, particularly *Electrona carlsbergi* and the squid *Kondakovia longimana*.

The isotope data suggest that this species feeds predominately on myctophids and other cephalopod species. However, stable isotope analysis could not be conducted on beaks that were smaller than 1.9 mm in lower rostral length (corresponding to a mantle length of 43 mm) as they had clear wings that had not been chitinized. Therefore, it is possible that this study has overlooked the predation of crustaceans such as amphipods and euphausiids by the smaller individuals in this population that had not been subjected to stable isotope analysis. This is likely prey for these smaller individuals, with squid being unable to predate on items that are larger than themselves, making it unlikely that they feed on larger myctophids (Rodhouse 1989).

4.1.4 Broad distribution of *Histioteuthis eltaninae*

The carbon isotope values demonstrated a relatively confined distribution to the waters that surround Macquarie Island for *Histioteuthis eltaninae*. There was no significant relationship occurring between size and $\delta^{13}C$ values, or between month and the carbon isotope signature. This demonstrates that all sizes at all times of the year inhabited the same confined distribution around the island. This also indicates that no migration occurred with size (and therefore age) or with season for this population of *H. eltaninae*. This implies that this population does not migrate to spawn and reproduce. Voss (1969) also came to the same conclusion, that *H. eltaninae* must be occupying the same vertical distribution and regions.

The $\delta^{13}C$ signature of *H. eltaninae* could be influenced by the distribution of the elephant seals that the beaks were obtained from. However, this appears to be unlikely with this species having such a confined distribution around Macquarie Island. The elephant seals that the beaks were obtained from foraged on a broad scale, ranging from the southern boundary of the Antarctic Circumpolar Current to the sub-Antarctic Zone (Field *et al.* 2004). Therefore, the foraging regions of the Macquarie Island elephant seals were unlikely to have an influence on the non-relationship between the $\delta^{13}C$ signature and size of *H. eltaninae*.

While these species inhabit the same region despite increased size and season, there is limited potential for competition for food resources between size groups in this population. With the positive relationship that occurs between size and trophic level, this results in larger sized individuals predating on higher trophic levels. While there may be competition occurring in other respects, it is likely that there is limited competition occurring in regards for prey items and what the different size classes are feeding upon.

When investigating *H. eltaninae* actual distribution, they are relative confined to a narrow range around Macquarie Island. It appears that *H. eltaninae* has a preference for lower temperatures and is limited by this factor to the subantarctic region (Voss 1969). It is likely that this species demonstrates a constricted thermo tolerance and therefore results in a restricted geographic distribution (Voss 1969).

Specimens have been collected between 92 to 2890 meters and it is likely that this species cannot tolerate higher temperature at their preferred depths outside of this sub-Antarctica region and it is this factor that restricts their distribution (Voss 1969). Therefore, this

would explain their narrow confinement in this current study as well as the absence of collection in research cruises such as the ELTANIN Cruise 28 that occurred in the Tasman Sea (Voss 1969).

4.2 *Martialia hyadesi*

Martialia hyadesi is a coldwater ommastrephid squid that is commonly found in the Macquarie Island region and has a broader circumpolar distribution around the Southern Ocean, particularly around the Antarctic Polar Frontal Zone (Arkhipkin and Silvanovich 2004; Dickson *et al.* 2004; van den Hoff 2004). Ommastrephid pelagic squid are generally related to frontal systems and in particular to eddy structures that occur at fronts, and *M. hyadesi* is no exception (Rodhouse *et al.* 1996). *M. hyadesi* has a known associated with warm core rings at the Polar Frontal Zone, and it is here that predation is believed to be favourable (Rodhouse *et al.* 1996).

Martialia hyadesi plays a significant role in the trophic food web of the Antarctic and is commonly found in the stomach contents of higher order predators, particularly southern elephant seals, Patagonian toothfish and albatross (Arkhipkin and Silvanovich 2004; van den Hoff 2004). There is an estimated consumption of 326,000 tonnes per year of *M. hyadesi* in the Scotia Sea alone by predators (Rodhouse *et al.* 1996). However, the stock size in this region is largely unknown and this appears to be the overall trend throughout the Southern Ocean for all cephalopod species (Arkhipkin and Silvanovich 2004; Rodhouse *et al.* 1996). However, the estimated annual consumption of a prey item such as *M. hyadesi* at least gives a minimum stock estimate, which is unavailable through conventional sampling surveys.

Martialia hyadesi is a common by-catch species of the *Illex argentinus* fishery at the Patagonian shelf and near South Georgia, particularly in colder years (Arkhipkin and Silvanovich 2004). These specimens yielded high returns and created an interest in a standalone fishery for the species. The first exploratory jigging trail occurred at the Polar Frontal Zone in 1989, which yielded high densities of this species (Rodhouse *et al.* 1996). There have been two exploratory fishing trails at South Georgia. The first occurred in 1996/97, where approximately 80 tonnes was captured (Kock 2000). The other occurred in

2000/2001, with the United Kingdom and Korea being successful in their application for an exploratory fishing expedition in this region (Kock 2001). It is also believed that there has been some illegal fishing attempts since the 1990s near the Patagonian shelf as the squid fishery begins to develop (Kock 2001). Therefore, knowledge into *M. hyadesi* life histories and their interactions with the ecosystem that surrounds them are essential for effective management. With cephalopods playing an important role in the diet of several high order predator species and their short-lived, semelparous life history, they have greater susceptibility to overexploitation, with far reaching ecological consequences if the fisheries is ill managed (Rodhouse and White 1995). Knowledge into the life histories of an exploited population in the Southern Ocean as well as that species interaction with the surrounding ecosystem is required by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR). A large consistent squid fishery of *M. hyadesi* occurs at the Patagonian and New Zealand shelf, straddling the region that is administered by CCAMLR (Kock 2000; Rodhouse and White 1995). This has the potential to complicate the management strategies of future fisheries that may occur for *M. hyadesi* in the Southern Ocean (Rodhouse and White 1995).

4.2.1 Size distribution of *Martialia hyadesi*

For *Martialia hyadesi*, there was a general increase in mantle length between February and December when investigating all of the beaks found in the stomach contents of elephant seals. Between September and December there were two overlapping size cohorts, which had some influence over this general increase in mantle length. The two simultaneous size classes over the four month period suggest a minimum life span of 16 month, with a new generation that is smaller and presumable younger entering the stock taken by the elephant seals in September. This species is semelparous, but demonstrates two spawning events during the winter, corresponding with the current study, and also during the spring period (Arkhipkin and Silvanovich 2004; Kock 2000).

The smallest squid eaten by elephant seals had a mantle length of 142 mm, but individuals smaller than 34 mm have been captured previously (Rodhouse *et al.* 1992a), This indicates that seals do not readily predate on smaller individuals or are potentially outside of their foraging range. Therefore, it is likely that this method of sampling the *M. hyadesi* population will underestimate the earlier life stages of this population to an extent.

Martialia hyadesi juveniles with a mantle length less than 34 mm are distributed over the eastern shelf at the Falkland Islands during October and November (Rodhouse *et al.* 1992a). This indicates that spawning occurs in the late austral winter or spring, which appears to correspond to the recruitment period in the present study.

The largest individual in this study had a mantle length of 485.41 mm, which is comparable to the largest specimen found in Rodhouse *et al.* (1996) captured in a pelagic trawl, which had a mantle length of 506 mm. This specimen was a female that had a maturity stage of I11 (preparatory) (Rodhouse *et al.* 1996). Considering that this specimen was not yet fully mature, this indicates that there are considerably larger individuals that have not been sampled yet. Therefore it would be safe to presume that *M. hyadesi* at Macquarie Island have a longer life span than 16 months as indicated by the stomach contents of the elephant seals. It appears likely that *M. hyadesi* has a life span closer to two years, as concluded by Piatkowski *et al.*(1991) for the population at Kerguelen Island. Rodhouse *et al.* (1996) believed that *M hyadesi* has a life span of more than one year, potentially up to two years. However, the life cycle and longevity of *M. hyadesi* is poorly understood.

4.2.2 Trophic position *Martialia hyadesi*

The positive linear relationship between $\delta^{15}N$ and size of *Martialia hyadesi* appears to be effected by season. However, it seems that the seasonal effect between January and March is due a combination of random sampling of the beaks and the fact that clear wings cannot be utilized in the stable isotope analysis. The mean size of January, February and April were larger for the stable isotope analysis when compared to the mean of all of the beaks for these three months. The greatest difference by more than 60 mm in mantle length occurred in January. The difference in mean size for January was due to smaller beaks having clear wings that could not be compared to larger darkened wings, due to varying chitin levels. The same principle applied for February and April also. In the months of January, February and April, the smallest beaks used to determine the nitrogen signature was 5.1 mm, 5.9, and 5.6 mm respectively. This was 3.6 mm larger than the smallest lower rostral length in January, and 1.15 mm larger than the smallest beak in April.

Another factor is that random selection selected larger individuals. This appears to be the case in January, with four of the five beaks having a lower rostral length between 10.5 mm

and 10.9 mm. In March the mean size of the beaks used in the stable isotope analysis had a lower mean size, and appears to be influenced by random sampling selecting beaks that were smaller, with five beaks having a lower rostral length between 3 and 5.3 mm. Despite the small mean size in the stable isotope analysis for March, the nitrogen signature was relatively high. The same occurred in February, but this month only had two specimens used for the stable isotope analysis.

Despite these factors, there is still a significant relationship for *M. hyadesi* between the size of the individual and the $\delta^{15}N$ signature. Individuals that had a mantle length smaller than 350 mm generally demonstrate a onestep trophic position of approximately 3 ‰. Generally, larger individuals demonstrate a greater $\delta^{15}N$ value, indicating a second trophic level. This conclusion is also enforced by the significant difference that occurred between the two concurrent size classes that occurred between September and December. These two size classes demonstrated an average trophic level difference. This indicates that while these two size classes occur at the same period of time, they are utilizing different prey resources, limiting competition for food.

4.2.3 Potential prey of *Martialia hyadesi*

There is a clear separation between the carbon and nitrogen stable isotope ratios of lower trophic levels and *M. hyadesi*, with the squid population being located further north than the potential prey items. The stable isotope data of this species did indicate a mixture of potential prey types. When the individuals are smaller (lower rostral length between 3 and 7.8 mm) prey appears to be dominated by euphausiid crustaceans and myctophid fish. The larger size class (lower rostral length between 7.8 to 11.6 mm) appear to predate on myctophids and squid. This diversity of prey leads to the general conclusion that *M. hyadesi* could be a generalist, opportunistic predator.

Generally, all of these prey items appear to align with previous studies into the stomach contents of *M. hyadesi*. The stomach contents of individuals captured in the north Scotia Sea indicated that the most frequent prey item was the myctophid fish *Krefftichthys anderssoni* and *Electrona carlsbergi*, the euphausiid *Euphausia superba* and hyperiid amphipods that was most likely to be *Themisto gaudichaudii* (Rodhouse *et al.* 1992b). Some were also cannibalistic and consumed other cephalopods (Rodhouse *et al.* 1992b). Several specimens have been captured at South Georgia on separate occasions. Rodhouse

et al. (1996), captured eight around the Polar Frontal Zone and found crustaceans, myctophid fish, other cephalopods and one specimen that contained the amphipod *Primino macropa* in its stomach contents. The amphipod, *P. macropa* is currently lacking in stable isotope composition and could not be compared in this current study. An exploratory fishery for *M. hyadesi* at north-west South Georgia also found that they largely predated on mesopelgic fish, crustaceans and other cephalopods indicated from their stomach contents (Dickson *et al.* 2004). Dickson *et al.* (2004) came to the conclusion that *M. hyadesi* was an opportunistic predator, which appears to aligns with the current study, particularly in regards to prey intake.

Myctophid fish appear to be a large component of the prey resources for larger individuals of *M. hyadesi* at Macquarie Island and also appeared to predate on other squid species such as *Histioteuthis eltaninae*. Predation on their own population as well as other cephalopod species appears to be a key feature of ommastrephid squid, particularly during migration and spawning events due to prey resources being limited (Dickson *et al* 2004). This has been found in the stomach contents of *M. hyadesi* individuals at South Georgia during the winter period when prey resources can be limited. Dickson *et al.* (2004) found that there was a high proportion of squid consumed, predominately *Gonatus antarcticius* as well as other *M. hyadesi*. This is contrasted to Rodhouse *et al.* (1992b), where cannibalism was not a major component to the diet of *M. hyadesi*. The contrasting outcomes in the stomach contents is likely to be a factor of prey availability, with the population sampled by Rodhouse *et al.* (1992b) likely to have sufficient prey available at the time of sampling. Cannibalism ensures the survival of at least a proportion of the population. When considering the current study, the carbon and nitrogen ratios of the larger individuals indicate predation on other squid species, which is likely the result of poor prey resources.

This study indicates a shift in diet from crustaceans to myctophid fish and other squid species as the population of *M. hyadesi* at Macquarie Island grow. This appears to be characteristic of ommastrephid squid (Rodhouse *et al.* 1992b). However, it is possible that this trend is confounded by the opportunistic nature of *M. hyadesi*, which would demonstrate seasonal and geographic changes in their diet. This species demonstrates a large geographical range and it has been found that there are differences in diet between locations (Dickson *et al.* 2004). These differences are likely to be the result of environmental variability of the prey availability rather than ontogenetic changes in their feeding (Dickson *et al.* 2004). Therefore it is possible that the shift in prey items with size

for the population at Macquarie Island is the result of their opportunistic predation. With extensive migration occurring in these individuals, the prey composition at the various locations will also differ. This will result in different potential prey items and therefore consumption by these individuals.

4.2.4 Broad distribution of *Martialia hyadesi*

There was a positive relationship occurring between the carbon isotope signature and the size of *Martialia hyadesi* individuals. This indicates that larger individuals occur further north, past Macquarie Island, but not quite to Australian waters, than smaller individuals, which occur further south to almost Antarctic waters, but not past the southern border of the Antarctic Circumpolar Current. However, while this relationship is significant, there is still an effect of season between January and April. The mean $\delta^{13}C$ value for February (-18.27 ‰) and March (-19.74 ‰) is approximately 2.5 ‰ and 1 ‰ further south respectively when compared to April (-20.75 ‰). When considering January (-19.11 ‰), this month occurred almost 1 ‰ further south when compared February, but approximately on par with March. February and March both had a smaller mean size of 6.95 mm and 5.32 mm in lower rostral length respectively in comparison to January (mean lower rostral length of 9.54 mm) and March (mean lower rostral length of 7.65 mm). This same trend also occurred in the $\delta^{15}N$ values for this species and a potential explanation is that the random sampling selected larger individuals during this period, with the exception of March, where smaller sized individuals appeared to be sampled. However, all of these carbon values for the four month period correspond to a broader distribution around Macquarie Island and never crosses the Polar Front.

The overall trend between the carbon isotope and the size of the squid indicates that smaller individuals of *M. hyadesi* in the southern Pacific Ocean occur in colder almost Antarctic waters, but do not cross the southern boundary of the Antarctic Circumpolar Current. As the individuals in this population get larger and therefore older, they migrate to warmer northern waters past Macquarie Island. This is also indicated by the two overlapping size classes in the spring and early summer. The individuals that had a mantle length less than 300 mm had a mean $\delta^{13}C$ value of -21.344 ‰, compared to the larger size cohort that had a mean $\delta^{13}C$ value of -19.25‰. However, both of these concurrent size classes were distributed north of the Antarctic Polar Front. This supports the conclusion

that the population actively migrates northwards as they age. Therefore they utilize resources at different locations at different stages of their life history, resulting in limited competition in this population.

A large proportion of juveniles *M. hyadesi* has been captured at the Falkland Islands, with mantle lengths smaller than 34 mm (Arkhipkin and Silvanovich 2004). This suggests that spawning occurs at the South Patagonian Shelf edge or the shelf break (Arkhipkin and Silvanovich 2004; Rodhouse and Piatkowski 1995; Rodhouse *et al.* 1992a). It has been suggested that the capture of immature individuals at the Patagonian Shelf, Scotia Sea and Macquarie Island are associated with islands or sea mounts, indicating that spawning occurs around these structures for *M. hyadesi* (Piatkowski *et al.* 1991).However, in the current study, these juvenile individuals were not represented, with the smallest individual having a mantle length of 142 mm, four times larger than the juveniles in Piatkowski *et al.* (1991). *Martialia hyadesi* paralarvae statoliths demonstrate fast growth, indicating that they hatch in relatively warmer waters (Arkhipkin and Silvanovich 2004). The life cycle of eggs, larva and juveniles are closely associated with the West Wind Drift, which transport these young individuals from warmer waters to colder, southern regions of the Southern Ocean (Piatkowski *et al.* 1991). Juveniles and maturing individuals demonstrate slower growth when compared to the paralarvae as indicated by the statolith increments, which would result from a cooler environment (Arkhipkin and Silvanovich 2004). Therefore, maturing *M. hyadesi* migrate southward during the austral winter to their feeding grounds that are distributed at the Antarctic Polar Frontal Zone (Dickson *et al.* 2004).

When considering the current study, juveniles were not captured by the elephant seals and therefore their distribution and migration cannot be determined. The lack of juvenile representation is possibly a reflection of the distribution of the juvenile elephant seals that the samples were obtained from. The smallest *M. hyadesi* individuals occurred in the austral winter period and during this season the juvenile seals only spent approximately 25 % of their sea time foraging in subantarctic waters (Field *et al.* 2004). *Martialia hyadesi* individuals are spawned in these northern warmer waters, where there was limited foraging time spent by their predators (Field *et al.* 2004). Hence, there is a lack of juvenile *M. hyadesi* individuals in the present study.

The individuals that are present indicated a migration pattern from southern, almost Antarctic water in the austral winter to northern waters beyond Macquarie Island during the austral summer. While this appears to contrast the literature, Arkhipkin and

Silvanovich (2004) predominately describe the migration of immature and juvenile individuals, which are lacking in this current study. Therefore, it is hypothesized that eggs, larva and juveniles originate in warmer northern waters that were then dispersed and migrate to southern colder waters. As indicated by this study, there is active migration by the maturing squid back to the warmer waters that originate north of Macquarie Island. The generalized life cycle of a ommastrephid species appears to be characterized by this migration cycle. Passive migration occurs down current to the feeding grounds where fast growth occurs and then these larger individuals actively undertake counter-current migration to northern waters where the spawning grounds are located (Rodhouse 1991).

4.3 Comparison of *Histioteuthis eltaninae* and *Martialia hyadesi*

While these two species are key prey resources to the elephant seal population at Macquarie Island, they are significantly different from each other in size, life span, distribution and diet. With *M. hyadesi* obtaining sizes more than five times larger than *H. eltaninae*, they are also able to achieve such sizes over a longer life span. *H. eltaninae* longevity appears to be approximately one year, where *M. hyadesi* has an estimated life span closer to two years. During their life time, *M. hyadesi* demonstrate a broader distribution both further south to almost Antarctic waters and north (almost Australian waters) than *H. eltaninae* who are thermally confined to regions in proximity to Macquarie Island. *M. hyadesi* are also opportunistic in their predation, feeding on crustaceans, myctophids and other squid species, where *H. eltaninae* appears to be specialist feeders on predominately myctophid fish. These two species have only one thing in common, and that is their importance in the ecosystem as a key prey resource to apex predators, particularly the elephant seals at Macquarie Island.

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Introduction

Throughout the marine environment, cephalopods have a significant impact on their surrounding ecosystem. This can be attributed to their biomass, with cephalopods having an estimated standing stock between 193 to 371 million tonnes worldwide (Stowasser *et al.* 2006). This allows them to be a diverse prey source for several marine top predators including marine mammals, seabirds and large pelagic fish. Such a high biomass of cephalopods also has a flow on effect to their own prey. They are able to be voracious, active and opportunistic predators due to their complex sensory structures and powerful muscular arms (Jackson 1993). These appendages are armed with suckers or hooks that are used to capture and immobilize their prey which can be as large as themselves (Rodhouse 1989).

In the Southern Ocean, a cephalopods importance is enhanced, with epipelagic fish being largely absent and having been replaced by the cephalopod community (Rodhouse and White 1995). The absence of epipelagic fish may the consequences of the physiological constraints that the Southern Ocean imposes on its inhabitants, such as the cold Antarctic waters, which it appears that they were unable to adapt to (Rodhouse and White 1995). Cephalopods life histories however, have several advantages which may not have constrained this group to adapting to this harsh environment. They demonstrate a short life cycle ranging from a few months to two years with fast growth during this time (Rodhouse and White 1995). They are also semiparous in their reproduction, allowing for a high investment in a large amount of offspring (Rodhouse and White 1995). Due to their short life cycle, the planktonic to nektonic phase (if present) are relatively brief, allowing them to stay in the productive regions before being flushed away by the Antarctic Circumpolar Current (ACC) (Rodhouse and White 1995).

However, despite the recognition of their importance in this environment, there is limited fundamental knowledge upon Southern Ocean cephalopod species. There are several reasons as to the lack of information into this group. Predominantly, though, it is attributed to the inadequacy of obtaining whole specimens by conventional sampling methods such as nets, due to the animals strong swimming capability (Nemoto *et al.* 1985). Such methods are floored with limitations in the Southern Ocean to begin with, with sampling only being able to occur during the ice-free summer periods, as well as the limited scientific surveys by research

cruises that are devoted to this group (Cherel *et al.* 2004; Cherel and Hobson 2005; Groger *et al.* 2000). There are also problems that arise due to the difficulty of collecting deep-water cephalopods, resulting in the lack of overall information about their biology and ecology for the region of the Southern Ocean (Ruiz-Cooley *et al* 2006).

Consequently, an alternative method of sampling was required and developed through utilizing their predators as a means of sampling the cephalopod population. Cephalopods have resistant, indigestible mandible (also known as beaks) which accumulate within the stomachs of their predators. While the analysis of stomach contents can yield information into the predator and ecosystem interactions, it can also provide considerable life history information into the cephalopod prey species themselves. Such investigations can be taken further with stable isotope analysis allowing for insight into their trophic position, possible prey resources and general distribution.

While such methods have been explored in various cephalopod predator species in the Southern Ocean, this knowledge has been relatively limited to the trophic links between squid species and their predators. Therefore there is a need to expand such methods further and focus upon cephalopods themselves in order to gain greater understanding of these species that play such a significant role within the Antarctic.

Identification of beaks

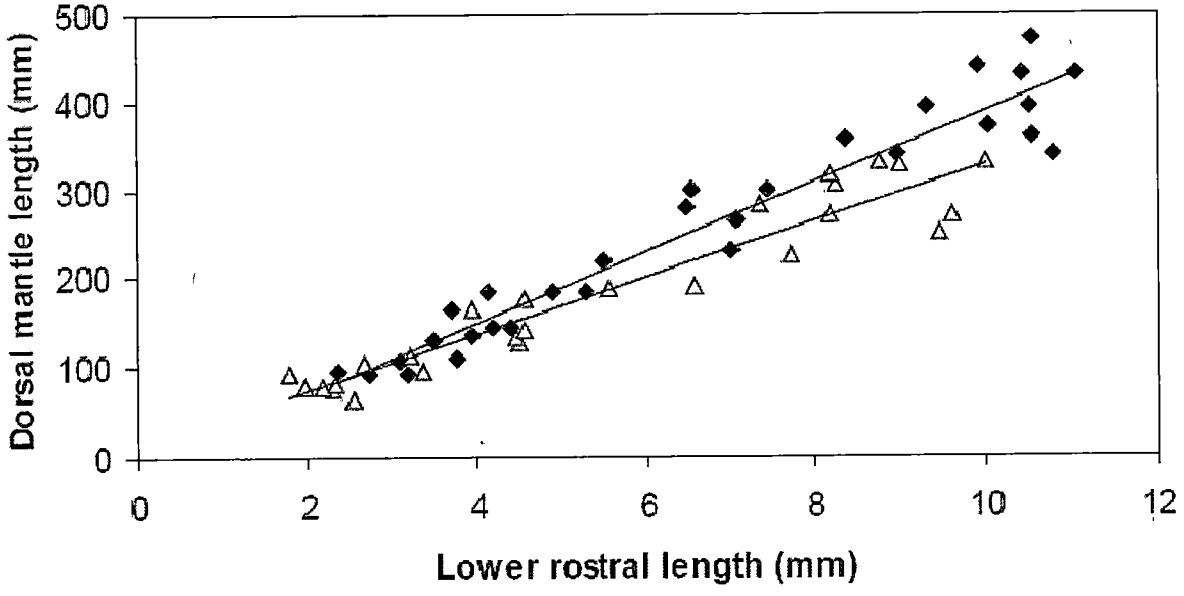
The use of beaks obtained from the stomach contents of their predators can be utilized as each species mouthparts are unique and can therefore be identifiable down to a species level. However, when looking at cephalopod beaks that have been obtained from the stomach contents of their predators there are several sources of bias that may occur. There are different retention times of a cephalopod beak in the stomach of their predators based on the size of the beak and its morphology. For beaks that are retained longer in the stomach, they are subjected to the digestion process for a longer period, causing increased erosion and decreased likelihood of identification (Santos *et al.* 2001). It is not unusual to be unable to identify a beak, with a great majority of studies including unidentifiable or immeasurable beaks (Xavier and Cherel 2009). This is predominately due to erosion and breakages of key identifying features. It can also be related to the fact that identification of cephalopod beaks is only possible when a species has been previously characterized from a complete animal (Xavier *et al.* 2007). As predators are far more efficient at catching cephalopods than conventional sampling method, there have been various cephalopod species that have yet to be described, limiting beak identification (Xavier *et al.* 2007). It is this same reason however, that makes the use of predators an opportunistic means of investigating such cephalopod species. If a large number of beaks from the same population can be identified, this can yield considerable results that would otherwise have been unknown.

Beak relationship with length and weight

The beak of a cephalopod can yield considerable information, with the dimensions of the mouth part having a strong relationship with the size of individual. The lower rostral length (LRL) is commonly used to determine the size of the individual, either in mantle length (ML) or mass. The literature commonly utilizes these relationships and the equations between the LRL and the size and mass of the animal are readily available in the literature for the majority of cephalopods (Groger *et al.* 2000). A positive linear regressions describe the relationship between the dimensions of the beak such as LRL and the mantle length (Lu and Ickeringill 2002). The relationship between the LRL and the mass of the individual is commonly a quadratic relationship. Such regression equations are species specific as the result of the beak morphology and dimensions being unique to a species, but not necessarily different between the sexes or as an individual matures, which can be seen in figure one (Bolstad 2006). If several individuals are present from the stomach contents of their predators, such measurements can yield estimates into the biomass of the cephalopod population that is consumed (Groger *et al.* 2000; Lu and Ickeringill 2002). This results in at least a minimum amount of biomass in a particular region, information that is currently lacking from conventional sampling methods. However, potential biases must be taken into account when estimating population size and biomass. Particularly in regards to the size of the individual (and therefore beak size), with there being varying retention time in the digestive tract of the predator depending on the size of the individual consumed. Larger individuals are generally retained in the stomach for a longer period of time, potentially biasing such estimates through over representing the larger individuals in the population. It is also possible that predators selectively target certain size classes and therefore do not accurately reflect the size distribution of a cephalopod population.

Retention time can also influence the investigation into the different populations of cephalopod species that occurs within a given region. This can be influenced by the same principles, in that different shape and sizes of beaks ingested can result in certain beaks being pass through the digestive system more easily than others (Xavier *et al.* 2007). Therefore over-estimations of the dominance and importance of a cephalopod population can occur in individuals that take longer to digest and therefore accumulate in a predator's stomach, resulting in bias.

Such biases should be considered and taken into account when investigating such broad cephalopod communities. In order to determine the possibility of the data being influenced by such means, cohort analysis conducted over the sampling period can indicate if there is an influence in the data. Frequency analysis of the LRL can determine if there is a skewedness towards smaller or larger individuals within the spread of the size over time and then this can be accounted for.



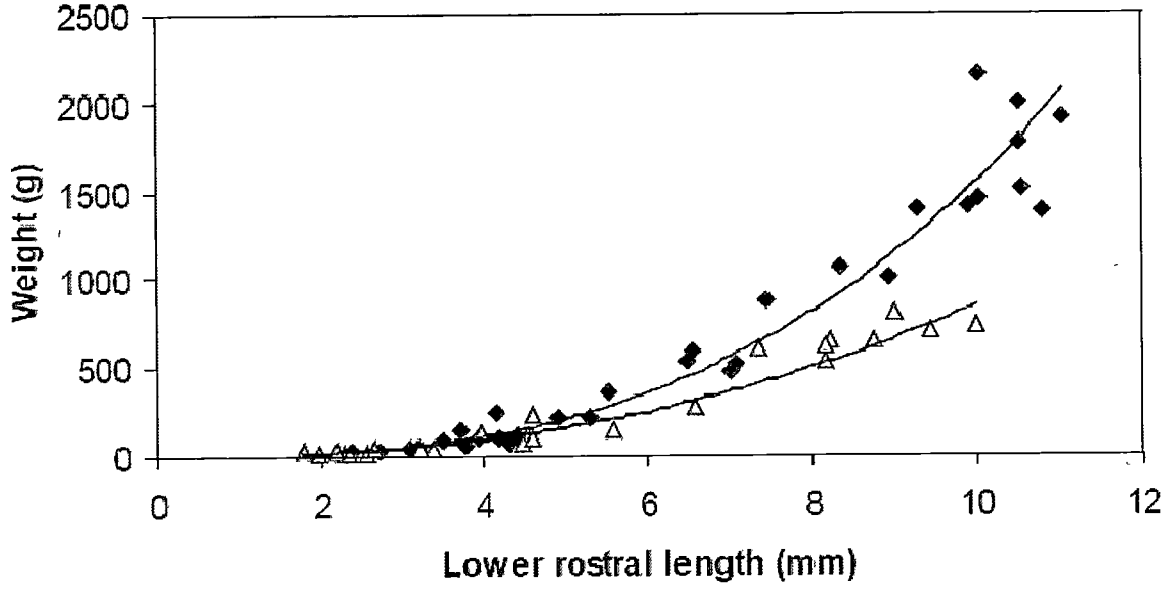


Figure 1: The relationship between the lower rostral length (LRL) and mantle length, and LRL and weight (wet preserved) for *Moroteuthis ingens*. The males are depicted as Δ, male (n = 30), □, female (n = 36) (Bolstad 2006).

Debate about length-frequency analysis

While the LRL can determine the length and weight of an individual, the use of such information may not be able to aid in determining a cephalopods life histories. Currently there is considerable debate about the use of length to presume a cephalopods age and therefore determine life histories such as growth and mortality. Originally squid growth was commonly estimated through length-frequency data by applying the von Bertalanffy function, a model that was developed to describe fish growth (Dawe and Beck 1997). However, since the 1980s, this technique has been questioned (Jackson *et al.* 1997). Questions were raised about this techniques when varying results were obtained from statolith ageing analysis when comparing it to the length-frequency analysis (Jackson *et al.* 1997). It is believed that length-frequency is inappropriate due to a cephalopods short life span (from a few months to two years) that demonstrate fast growth during this time, causing a high turnover rate of generations (Oosthuizen 2004). Their growth is also strongly influenced by several biotic and abotic factors, predominately water temperature and food availability (Oosthuizen 2004). Their short life span results in environmental factors causing different growth rates between each of the cohorts. This plasticity in their growth allows cephalopods to take advantage of patchy resources, resulting in a boom of growth when conditions are favorable. However, it is these same factors that cause cephalopods to be unable to store reserves which are needed to survive infertile periods, resulting in a crash of the cephalopod population during these times (Jackson *et al.* 2005). The combination of a cephalopods short life span, extended reproductive events as well as their rapid growth rates are closely correlated to environmental features causes a complex population structure that can often be unpredictable (Jackson *et al.* 2005). The consequence of these factors is large variations in growth between population and has been observed in many cephalopod species (Oosthuizen 2004). However, variability of a cephalopods growth is not just limited to larger scale differences on a temporal and spatial scale. Different growth rates can be observed in a given population. With a prolonged spawning period that is demonstrated in many cephalopod populations, environmental conditions differ for those that are born at the start of the season when compared to those that emerge at the end of the spawning period. Therefore, their growth rates within a given cohort will differ causing the estimating age from the size of a cephalopod difficult. This means that Lee's phenomenon applies to cephalopod populations, where faster growing individuals have a higher mortality rate than that of slower growers. These varying growth rates confounds the effects of immigration and emigrations (Rodhouse and Hatfield 1990). It is believed that high migration does occur in squid populations, but are difficult to quantify due to this variability in their growth, enhancing the inappropriateness of length frequencies for cephalopods (Dawe and Beck 1997).

However, despite the mounting evidence, papers continue to publish length-frequency analysis for species of squid. This is despite the evidence that both finfish models and length-frequency analysis are inappropriate for squid and that such techniques will result in profound inaccuracies in the estimation of squid growth and their life span (Jackson *et al.* 1997). Such

models and analysis causes an overestimation of the life span of cephalopods and therefore underestimates their growth rates. Length-frequency analysis should therefore not be used in determining the growth rates squid (Jackson *et al.* 1997)

Ageing beaks

With the use of length-frequencies as an indication of age for cephalopods being a questionable technique, other methods of accurate ageing are required. While statoliths are the most common structures used in the determination of age within squid species, there are several inadequacies with the use of this method. Firstly, that this structure is only available in whole squid specimens and not readily available in stomach contents. This means that conventional methods need to be implemented to obtain whole individuals. However, in the Southern Ocean such methods are inappropriate. The search for alternative structures to aging cephalopods have included the analysis of periodic deposition on different hard structures such as the beaks, radulae, gladii, cuttlebones, and eye lens (Bettencourt *et al.* 1996; Bettencourt and Guerra 2001). The ageing methods that use hard remains are based on the study of the incremental growth structures which are considered to be the most appropriate for cephalopods (Perales-Raya *et al.* 2010). Studying the increments in these calcified structures are not limited to establishing the age of an individual cephalopod. Through accurately ageing these individuals, the life histories of a population can be determined. Such information includes growth rates and growth modeling, cohort analysis, when maturation occurs, hatching dates, migrations and ecological information of a particular population (Bettencourt *et al.* 1996).

Beak structures are believed to be one of the most promising of these hard structures. This is the result of beaks being largely resistant to digestion in the stomachs of their predators. Therefore they are usually the only remains of the squid in the stomach contents of their predators that can yield such ageing data. Hence, stomach contents could be utilized in yielding significant amounts of information into the life histories of these cephalopod species. Beaks are composed of a chitin-protein complex and secreted by a single layer of tall columnar cells that are known as beccublasts that are responsible for their growth (Perales-Raya *et al.* 2010). The chitinization and hence the growth of the beak process starts at the rostrum tip and then gradually builds to the lateral wall and then to the wings (Perales-Raya *et al.* 2010). This results in the rostrum tip being the oldest region of beak, with the wings being the newest structure formed, with these structures being seen in figure two. While authors have reported rings observed in beaks, there has been limited attempts to use these structures in ageing of cephalopods, let alone to validation of the hypothesized one-increment representing one-day (Oosthuizen 2004).

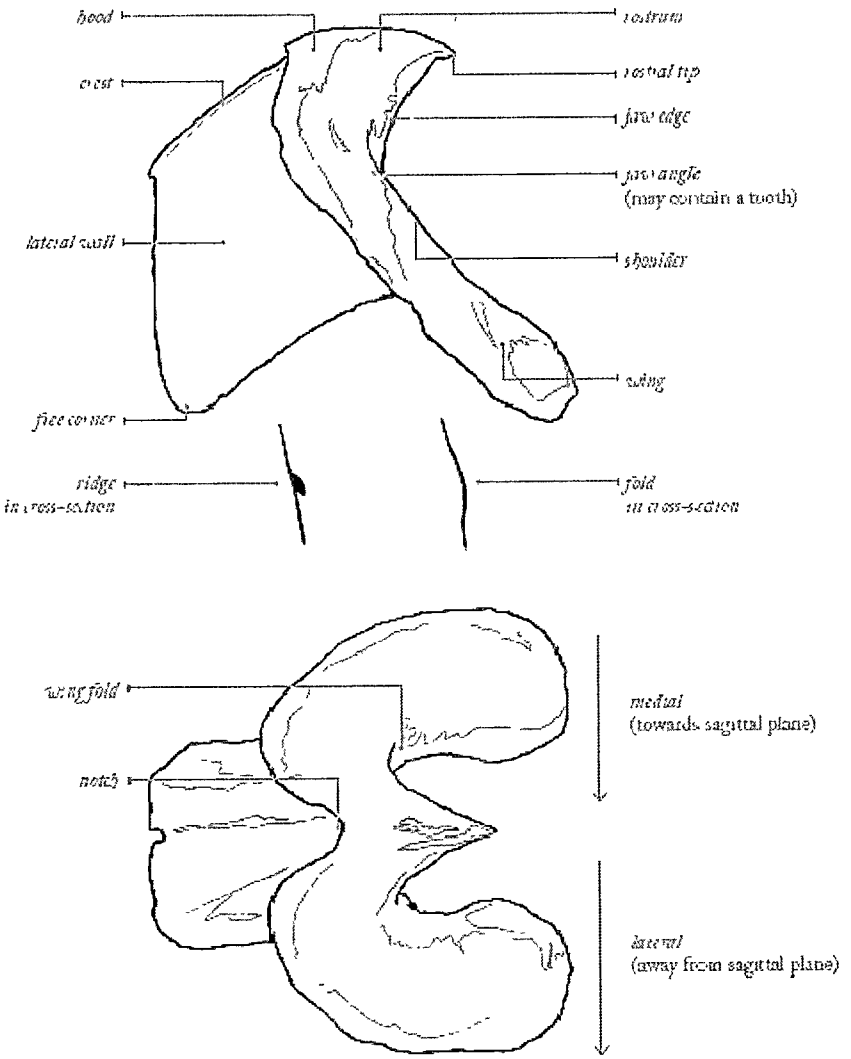


Figure 2: Key features and the principal terms used to characterize decapod beaks (Xavier and Cherel 2009)

Tetracycline is a chemical marker that was used to stain statoliths in order to validate the one-day, one-ring deposition hypothesis that was once speculated about and was successfully proven (Oosthuizen 2004). Preliminary results using this chemical marking in beaks have been successful and demonstrated a daily deposition of increments of adults of a common octopus species, *Octopus* vulgaris, however, definitive validation is still necessary (Perales-Raya *et al.* 2010). The ring formation in cephalopods beaks in determining the age of an individual has been likened to the use of statoliths within squid ageing and appears to be a promising technique. Particularly when compiled with the use of stomach contents in providing the beaks of which to age such individuals and populations.

However the use of beaks still has its downfalls, with possible erosion of the rostral tip throughout the life of the individual. This erosion may bias the age determination in underestimating the age of the individual and therefore has to be taken into account (Perales-Raya *et al.* 2010). One potential mitigation procedure is to determine the distance between the individual increments, and then to extrapolation how many increments should be inhabitating this eroded region. This potentially will allow for a more accurate representation of the age of the cephalopod individual.

Stable isotope analysis

The beaks obtained from the stomach content of predators can also be subjected to other methods of analysis. One such method is stable isotope analysis, which commonly determines the nitrogen ($^{15}N/^{14}N$, measured as $\delta^{15}N$) and carbon ($^{13}C/^{12}C, \delta^{13}C$) ratios in the sampled structures. This method is increasingly used to complement stomach content analysis, allowing for the investigation of the food web structures in the marine ecosystem and on the species themselves (Ruiz-Cooley *et al.* 2006). Such isotopes can be measured due to the fact that inorganic substrates (such as carbon and nitrogen) are incorporated into primary producers by the photosynthesis processes. However, the carbon and nitrogen isotopes values vary spatially due to primary producers being regional different due to environmental factors and growth rates (Porras-Peters *et al.* 2008). Such influencing factors include nutrients, light levels, type of primary producers, the isotopic composition of the substrates, the intensity of upwelling and the magnitude of atmospheric inputs (Porras-Peters *et al.* 2008). The theory behind this method is that stable isotopes elements enter the food web during primary production from inorganic substrates including nitrogen and carbon (Hobson and Cherel 2006). Therefore their behaviour through the trophic levels of an ecosystem can be predicted (Hobson and Cherel 2006). By knowing how the isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$) change during this process, it is possible to forensically delineate the source of the individuals prey and therefore its trophic position within the food web (Hobson and Cherel 2006).

$\delta^{15}N$ levels demonstrate an average stepwise enrichment of 2.5-3.4 ‰ with each of the changes in trophic level (Cherel and Hobson 2005; Stowasser *et al.* 2006). The differences within the $\delta^{15}N$ levels between individuals allow for the assessment of a species' position in a food web.

$\delta^{13}C$ levels however, vary little along the food chain (Stowasser *et al.* 2006). Therefore $\delta^{13}C$ is usually used to determine the broad distribution of an individual. This is due to higher latitude plankton food base (such as phytoplankton and particulate organic matter (POM)) being more enriched with $\delta^{13}C$ when compared to the equatorial community (Cherel and Hobson 2007). In the southern hemisphere, the geographical $\delta^{13}C$ gradients are well defined with high $\delta^{13}C$ values in the cold southern Antarctic waters and decreasing in $\delta^{13}C$ as you

move towards the tropics, and can therefore be use for the investigation of foraging regions of marine predators (Cherel and Hobson 2007). However, it has been suggested that $\delta^{13}C$ values of surface POM in oceanic waters are not linearly related to latitude (Cherel and Hobson 2007). Instead, the latitudinal changes are stepwise, with little deviation with POM carbon signatures in a given water mass and abruptly changes at the fronts (Cherel and Hobson 2007). Therefore, if this concept is correct, there is a relatively consistent value found across the Antarctic Zone with fairly steep increases across the Polar and Subantarctic Front and the Subtropical Front (Cherel and Hobson 2007). The spatial accuracy of the determination of the foraging region of consumers that use $\delta^{13}C$ values operate at a large geographical scale of water masses and fronts (10s to 100s of km in the latitude) (Cherel and Hobson 2007).

It has been demonstrated that the use of stable isotopes on squid beaks is a useful approach in the investigation of feeding ecology of cephalopods (Ruiz-Cooley *et al.* 2006). The relative trophic position of a cephalopod can be inferred through the use of the break (Ruiz-Cooley *et al.* 2006). Since the beak is an inactive tissue, it can reflect an individual's diet throughout their growth, with the isotope composition of the most recent growth bands in a cephalopod beak indicating the isotopic signal of their more recent diet (Ruiz-Cooley *et al.* 2006). This is the wing and lateral wall structure seen in the cephalopod beak. This is due to a cephalopods beak growing through the accretion of new molecules of proteins and chitin and having no turnover after their synthesis (Cherel and Hobson 2005). Consequently, the cephalopod beaks retain molecules that builded up from their early development to the time of their death and their isotopic signature is integrated from their feeding ecology over its whole life span (Cherel and Hobson 2005).

However, there are biological variables such as age, body condition, maturity, water stress, body protein and urea recycling that can have a significant impact upon the stable isotope ratios and therefore their interpretation (Dehn *et al.* 2007). The findings within Stowasser *et al.* (2006) suggest that fractionation and deposition of nitrogen and carbon isotopes in body tissues are strongly dependent upon metabolism and the general condition of the organism at any stages within their life history (Stowasser *et al.* 2006).

There are other limitations to stable isotope analysis, in that it offers less detailed information upon dietary composition, such as the species that have been predated upon, which stomach contents and scat analysis can provide. However, it does provide information upon their trophic level, which can then be extrapolate to determine their food resources and avoids some of the biases of other conventional methods such as stomach content analysis (Porras-Peters *et al.* 2008).

Southern Ocean fisheries require this information

The beaks that are obtained from the stomach contents of a cephalopods predator can yield considerable amounts of information into a population that have previously been lacking in any knowledge. Predators are able to sample populations and size classes that are out of reach of human sampling, particularly in the Southern Ocean. These beaks can give life history knowledge as well as determining broad distribution and trophic position as well as potential prey resources that would have otherwise been unknown. Such information is not only vital in regards to the cephalopod population themselves, but are also need in determining how these populations function on an ecosystem scale. In the Southern Ocean, this is of vital importance when considering that cephalopod fisheries are approaching the boundaries of the Southern Ocean.

With increasing populations around the world, there has also been an increase in pressure to expand the world's fish catch. With the pressures upon fish stocks continuously increasing through time, and conflicts arising between fishing nations, there is a need to expand to other species to harvest as well as other regions (Rodhouse and White 1995). This is particularly evident with currently exploited finfish and cephalopod stocks failing to provide a sustainable yield or declining, causing a growing interest from fishing nations to find new and unexploited stocks (Rodhouse 1989). From the 1980s, fishers have turned their attention to squid harvesting and the potential of utilizing Southern Ocean stocks (Rodhouse 1989). Major fisheries of cephalopods that have been developed in the waters that surround New Zealand and the Falkland Islands, enclosing on these unexploited stocks of the Southern Ocean (Rodhouse 1989). In both the New Zealand and Falkland Island fisheries, the Japanese, Koreans and the Taiwanese have dominated these fleets (Rodhouse 1989). However, in the Falkland Islands fishery they have been accompanied by vessels from a number of European countries, particularly Spain as well as the Eastern Bloc, particularly Poland (Rodhouse 1989). There have also been some exploratory fishing trails that have occurred south of the Antarctic Polar Front (APF) in the Scotia Sea by the Japanese and the Russians and it is likely that there will be further exploration and interest in the region (Rodhouse 1989). Such interest has been focused on the Southern Ocean due to an increasing awareness of the presence of very large populations of these animals to the south of the Southern Sub-Tropical Convergence (SSTC) and the recent growth of cephalopod fisheries around the world that has also seen their market value increase (Rodhouse 1989).

The ommastrephid *Martialia hyadesi* has the greatest potential for harvest in the Southern Ocean, with stocks being utilised in the high seas north of the region that is managed by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (Rodhouse and White 1995). There is also potential for a fishery of this species in the South Georgia region which is presumed to have a large stock due to the large community that predates upon them (Rodhouse 1990). This species also occurs as a by-catch from the *Illex argentinus* fishery on the Patagonian Shelf but with variable catch outcomes (Rodhouse 1990).

Within 1986, 22 000 tonnes were caught on the Patagonian Shelf by Japan (Rodhouse 1990). Within the previous years the catches were noticeably less and in 1987 only 20 tonnes of this squid were reported in the Falklands Interim Conservation Zone (FICZ) (Rodhouse 1990).

In the 1990s the market value for *M. hyadesi* approaches that of *I. argentinus* (Rodhouse 1990). With the price increasing for cephalopods, fishing vessels may start to focus on southern waters such as the Scotia Sea in search of *M. hyadesi (*Rodhouse 1990*)*. Other possible commercially exploited species of onychoteutids, *Kondakovia longimana*, *Moroteuthis ingens*, *M. knipovitchi* and *M. robsoni* and the gonatid, *Gonatus antarcticus* (Rodhouse 1990). They are members of families that are subject to direct fisheries around the world or are caught as by-catch but still have commercial value (Rodhouse 1990). While there are no known fisheries for the members of the Brachioteuthidae, Psychroteuthidae or Neuteuthidae, their flesh appears to be suitable for human consumption, and are caught in large enough quantities that they may contribute to a commercial fishery (Rodhouse 1990).

If these potential cephalopod fisheries become a reality, it will be necessary to develop management strategies to ensure the sustainable harvest in regards to individual populations and also provide a tool for which effort of fishing is controlled (Stowasser *et al.* 2006). In order to develop such management aims for a squid species, detailed knowledge of their trophic relationships, particularly predation, are required as there may be a significant influence in the community structure and the population dynamics of their ecosystem if exploited too heavily (Stowasser *et al.* 2006). However, such information is presently lacking and if exploitation was to be proposed, then management strategies require adequate knowledge upon the exploited species and their impact upon the surrounding ecosystem.

However, normal fisheries management shortcomings are complicated further with cephalopods in regards to their life histories. With cephalopods generally being semelparous and having a short generation time, they are more prone to extreme fluctuations in population size. They are therefore particularly susceptible to overfishing as the result of recruitment being dependent upon the breeding success of a single exploited generation (Rodhouse 1990). Such fluctuations will be even more pronounced within the Southern Ocean populations, with the environmental variables being greater as well as resources being patchy and inconsistent throughout time and space.

It should also be recognised win management strategies that fishing practices select particular traits within their target species (Rodhouse 1990). Therefore life history traits may be susceptible to selection-induced genetic changes (Rodhouse 1990). If there are genetic changes within the cephalopod population as the result of artificial selection imposed by fishing gear, changes within the mean growth rate or spawning time might become apparent in a relatively short time after exploitation begins due to their short generation time (Rodhouse 1990). The long-term consequences of fishery-induced genetic changes could be considerable if the fishing is intense and the stocks are geographically isolated (Rodhouse 1990).

Not only do fisheries affect the species that are being harvested, but with poor management and overexploitation, it can have detrimental consequences throughout the ecosystem. Therefore fisheries management practices need to understand the relationships between the harvested species and other top-level predators (Hindell *et al.* 2003). If such knowledge is unknown when fisheries are allowed, this is likely to produce unpredictable outcome on lower trophic levels due to the complicated and largely unpredictable outcomes that result from altering the populations size of a secondary producer (Hindell *et al.* 2003). This predicament is particularly prominent in the Antarctic and the Southern Ocean marine ecosystem where there is a low diversity of secondary producer species although these few species occur in a vast quantity (Hindell *et al.* 2003). CCAMLR is responsible for the management of fisheries within the Antarctic region and tries to do so from an ecosystem perspective, through maintaining the ecological relationship and integrity between the harvested, dependent and competing populations (Hindell *et al.* 2003). While there has been several studies investigating Antarctic predators within the Southern Ocean and attempts to model the potential impacts of fisheries upon local ecosystems, the majority have been based upon limited samples and temporal restrictions (such as during specific times of the year) (Hindell *et al.* 2003). There has also been no attempts to assess how representative such samples are of the entire population (Hindell *et al.* 2003).

If cephalopods were unmanaged or ill managed in regards to fisheries management in the Southern Ocean, there is the potential of severe and wide reaching effects not only on their own population, but on their surrounding ecosystem that relies upon such stocks. They are a major component in the diet of several species of higher order predators in the Antarctic and are particularly vulnerable to overfishing due to their short life span that is semelparous (Rodhouse and White 1995). Therefore, basic life history knowledge and the cephalopod stocks interactions with the rest of the ecosystem must be quantified before such fisheries and their management can be determined and harvesting of these species begins.

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